STEWART, ISAAC H., M.S. Development of Real-Time PCR to Identify Cyanobacteria Populations in Lakes. (2011)
Directed by Dr. Parke Rublee. 59 pp.

The objective of this project was to develop and test primers for real-time PCR analysis of cyanobacteria. Primers were developed using an existing set of sequence libraries from the variable regions of the 16S rDNA for specific operational taxonomic units (OTUs) and then validated against existing clones and previously identified pure cultures. Environmental samples from High Point City Lake were then analyzed as a practical test to assess cyanobacterial abundance and growth patterns. Of 96 OTUs identified from the 883-sequence library, 30 OTUs were probed for in 18 genomic DNA samples collected from December 2007 through December 2008. OTU presence was confirmed and DNA was quantified from standard concentrations. OTUs were most abundant during summer months when water temperatures were highest. This study suggests that real-time PCR is an accurate way to monitor cyanobacteria diversity in City Lake. Given the conservation of 16S rDNA, climate change, increasing eutrophication of waterways, and general cyanobacteria abundance, this method of assessing cyanobacteria diversity is a step toward developing a rapid and efficient way to monitor the increasing abundance of cyanobacteria species and strains in water bodies.

DEVELOPMENT OF REAL-TIME PCR TO IDENTIFY CYANOBACTERIA POPULATIONS IN LAKES

by

Isaac H. Stewart

A Thesis Submitted to
the Faculty of The Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Greensboro
2011

Approved by

_____
Committee Chair

APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

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Date of Acceptance by Committee

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Date of Final Oral Examination

ACKNOWLEDGEMENTS

I would like to thank all of the people who have helped on this project. Dr. Parke Rublee, without whose help, I would still be lost. The members of my committee, Dr. Dave Remington and Dr. Vince Henrich, who have been exceedingly flexible with my defense schedule. Bill Frazier, who was gracious enough to work with our lab and the NCSU Center for Applied Aquatic Ecology (CAAE) by allowing access to City Lake. The environmental data and samples were collected by researchers at the NCSU CAAE. To Mike Marshall, who generated the sequence libraries I used in this study and tutored me in primer design. Funding support for this project was provided by EPA STAR grant RD-83162701 and UNC Competitiveness Research Funding to Parke Rublee and Vincent Henrich, and UNCG Department of Biology Graduate Research Funding.

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CHAPTER I

INTRODUCTION

Cyanobacteria occur in marine and freshwater resources ranging from small eutrophic farm ponds to large areas of oceans (Fristachi *et al.*, 2008). Periodic increases in growth occur, resulting in high biomass events known as blooms. Cyanobacterial blooms are found more often and last longer in areas with warmer climates (Fristachi *et al.*, 2008). Interactions between hydrology, nutrient loading, sunlight, temperature, and ecosystem disturbance, among other things, often result in the occurrence of blooms. The nature of these interactions and the connection between systems makes identifying specific causal agents difficult. Nitrogen and phosphorus loading are generally presumed to be more direct causes of blooms and as such have been the focus of studies trying to control blooms (Perovich *et al.*, 2008).

Human-induced eutrophication of aquatic systems has caused a reduction in species diversity and richness of aquatic macroinvertebrates, fish, and primary producers (Paerl and Huisman, 2009). Human engineering of aquatic systems has also affected species diversity. For example, run-of-river impoundments create selective habitats for primary producers (Baldwin *et al.*, 2010). Green algae dominate riverine sections of reservoirs. Lacustrine sections of reservoirs show increased stratification which encourages cyanobacterial growth. In general, poor water quality results in the visible occurrence of cyanobacterial blooms. High nutrient concentration is a direct cause of these blooms.

Many cyanobacteria are capable of nitrogen fixation which occurs most significantly during warm seasons (Doyle *et al.*, 2010; Erkaya *et al.*, 2011). This suggests that phosphorus is the limiting nutrient in cyanobacteria growth. Another limiting factor in cyanobacteria growth is light intensity. It has been shown that phytoplankton biomass can be reduced by a high non-algal turbidity, with secchi depth less than 2.02 m^{-1} (Dzialowski *et al.*, 2011).

Cyanobacteria are being studied worldwide (Fristachi *et al.*, 2008; Akcaalan *et al.*, 2009; Harding *et al.*, 2009; Erkaya *et al.*, 2011; Fathalli *et al.*, 2011; Xu *et al.*, 2011). Scientists suggest that as global climate changes; cyanobacteria will dominate phytoplankton communities, for several reasons (Paerl and Huisman, 2009). First, high atmospheric CO_2 content will result in higher rates of photosynthesis. This carbonate source can change the pH of water, allowing more tolerant cyanobacteria to out-compete other phytoplankton. Second, as water temperature increases, phytoplankton growth rate increases. While eukaryotic primary producers' growth rates begin to decline when water temperature reaches 25^{o} C, cyanobacterial growth rates remain high. Finally, with unknown weather patterns associated with climate change, droughts that increase salinity of waters can encourage growth of salt-tolerant cyanobacteria (Paerl and Huisman, 2009).

While cyanobacteria can have long-term effects on global water supplies, they also can significantly impact freshwater supplies on a more local and short-term scale. Large cyanobacterial blooms increase biological oxygen demand, through respiration as well as decomposition. This demand has been shown to cause anoxic and hypoxic conditions and subsequent fish kills (Fristachi *et al.*, 2008). Decomposition of cyanobacterial

blooms has also been shown to have drastic effects on water chemistry, most notably rapid increases in pH as a result of increased ammonia content (Bury, 2007). Changes in the benthic plant communities can occur when blooms form large mats at the surface of a water source and block sunlight from reaching the benthos. These mats can also serve as a heat-sink for sunlight, potentially elevating surface water temperatures to well over 40^{o}C (W. Frazier, City of High Point Water Quality Laboratory. - personal communication). Concentrated cyanobacterial blooms can also create odor and taste problems in drinking water reservoirs, which negatively impacts recreation and may increase treatment costs (Fristachi *et al.*, 2008). It is impractical for water treatment facilities to remove these compounds continuously (Dzialowski *et al.*, 2009). Alternatively, physical or chemical removal of cyanobacteria is also costly. Treatment costs for cyanobacteria range from $1247 to $19227 per hectare for mechanical harvest, which can only be used for filamentous or large morphologies (Dodds *et al.*, 2008). Chemical treatment costs range from $246 to $1190 per hectare (Dodds *et al.*, 2008).

Cyanobacterial Toxins

Cyanobacteria are also a real and present concern in our waterways because of the toxins that they are known to produce (Table 1). Organisms capable of producing toxins only do so under appropriate conditions; thus, presence of the organisms does not necessarily indicate toxin production (Furukawa *et al.*, 2006). Environmental variables

Table 1. Toxins produced by cyanobacteria. (after Carmichael, 2001; Fristachi *et al.*, 2008)

Hepatotoxins	**Genera of Producers**
Microcystins	*Anabaena, Haphalosiphon, Microcystis, Nostoc, Oscillatoria, Phormidium, Planktothrix*
Cylindrospermopsin	*Aphanizomenon, Cylindrospermopsis, Raphidopsis, Umezakia*
Nodularin	*Nodularia*
Neurotoxins	
Anatoxin-a	*Anabaena, Aphanizomenon, Cylindrospermum, Microcystis, Oscillatoria, Planktothrix, Raphidiopsis*
Anatoxin-a(S)	*Anabaena, Aphanizomenon, Planktothrix*
Paralytic Shellfish Poisons (Saxitoxins)	*Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Phormidium, Trichodesmium*
B-N-methylamino-L-alanine (BMAA)	*Anabaena, Cylindrospermopsis, Lyngbya, Microcystis, Nostoc, Phormidium, Planktothrix, Plectonema, Prochlorococcus, Synechococcus, Tricodesmium*
Avian vacuolar myelinopathy (AVM)*	*Stigonematales*
*AVM is a neurological disorder in birds that is suspected to be linked with ingestion of toxin-producing cyanobacteria. The cause is still unknown.	
Dermatotoxins	
Lyngbyatoxin-A	*Lyngbya*
Aplysiatoxin	*Lyngbya, Oscillatoria, Schizothrix*
Endotoxins	
Lipopolysaccharides (LPS)	*Aphanizomenon, Oscillatoria, Schizothrix*

may stimulate cyanotoxin production and cell death can cause the release of toxins into the water (Bury, 2007).

The most common cyanotoxin associated with cyanobacteria is the potent hepatotoxin microcystin. It is also the most well studied cyanotoxin. More than 60 variants of microcystin have been identified (Hisbergues *et al.*, 2003). Chronic low-dose exposure to microcystins has been shown to promote the growth of liver tumors in a laboratory setting (Humpage and Falconer, 1999; Chorus *et al.*, 2000).

A large cluster of genes (*mcy*) encoding tailoring enzymes, peptide synthetases and polyketide synthases, is responsible for the synthesis of microcystins (Hisbergues *et al.*, 2003). There are regions in the *mcy* cluster that allow for the selective identification of microcystin-producing cyanobacteria in various genera using PCR (Hisbergues *et al.*, 2003). Furukawa *et al.* (2006) suggest that real-time PCR can be used to estimate *potential* toxicity in environmental samples based on DNA from microcystin-producing cells, but this method cannot be used to confirm the presence of the toxin. For rapid testing in the field, "dip sticks" have been produced to do on-the-spot microcystin testing. These immunochromatographic lateral-flow assays use red-colored antibodies for visual microcystin detection (Harding *et al.*, 2009; Tippkötter *et al.*, 2009).

While hepatotoxins are broadly studied, other toxins including recently discovered neurotoxins are associated with cyanobacteria (Cox *et al.*, 2005; Wilde *et al.*, 2005). Certain species of *Nostoc* have recently been shown to produce B-N-methylamino-L-alanine, BMAA, which has been linked to the amyotrophic lateral sclerosis / Parkinsonism–dementia complex, ALS/PDC. *Nostoc* symbiosis in the roots of *Cycas micronesica* results in biomagnification of BMAA from cycad seeds to flying foxes, which are eaten by natives of Guam (Cox *et al.*, 2005), where there is a "hotspot" of neurological disease. Another recently documented neurological disorder potentially linked to cyanobacteria is avian vacuolar myelinopathy (AVM). Cyanobacteria of the genus *Stigonematales* growing as epiphytes on invasive aquatic weeds are suspected to produce toxins that cause brain lesions in birds that eat them. The neurological

symptoms associated with AVM make afflicted waterfowl targets for raptors and other birds of prey, for example, AVM-attributed deaths of bald eagles (Wilde *et al.*, 2005).

Dermatotoxins and endotoxins are additional cyanobacterial toxins (Table 1). The dermatotoxins lyngbyatoxin A and aplysiatoxin have both been associated with skin irritation or "swimmer's itch." They also are known to interfere with protein kinase C and cause tumors (Ito *et al.*, 2002). Like many other gram-negative bacteria, cyanobacteria have endotoxins in their cell walls. Endotoxins may enter the body and associate with toll-like receptor-4 triggering inflammation and endotoxic shock (Madigan and Martinko, 2006). In fish, this lipopolysaccharide-induced endotoxic shock causes the heart rate to rise and may result in fish kills (Bury, 2007).

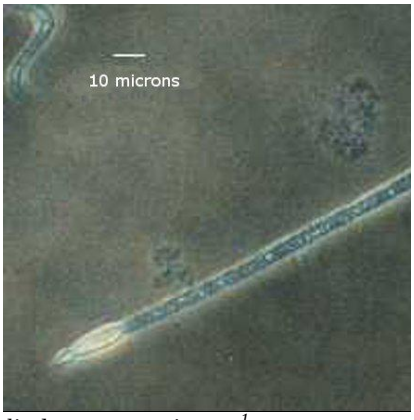
Cyanobacteria Identification

Traditional classification techniques for cyanobacteria focus on morphological characteristics of colonies and individual cells (Figure 1). Some cyanobacteria are difficult to culture, which makes taxonomic identification difficult, and suboptimal culture conditions can also lead to alteration of morphological characteristics. As a result, ideal identification of cyanobacteria is done with *in situ* populations rather than in laboratory cultures, but identification between species with similar morphologies can be difficult (Nubel *et al.*, 1997). Emerging molecular techniques offer another method of identifying cyanobacteria to complement morphological traditions.

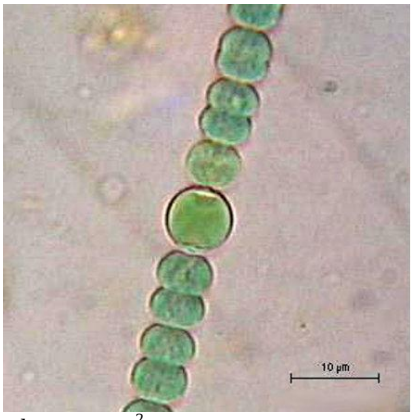
Research in molecular ecology has allowed for the development of diverse tools and protocols useful for identifying organisms based on their molecular characteristics. It is generally accepted that ribosomal RNA is highly conserved within species (Nubel *et al.*, 1997). Using 16S rRNA gene sequence analysis provides identification that is independent of the morphological differences that may occur between field and culture conditions (Nubel *et al.*, 1997). In order to effectively use PCR-based molecular approaches for field identification, gene sequence information is necessary. Classification of cyanobacteria based on the 16S rRNA gene is limited by available sequences, but the number of available sequences is increasing.

Alternative molecular tools useful in identifying cyanobacteria also have their basis in DNA sequence analysis. Denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis have been used to isolate proteins and nucleic acid sequences based on charge, conformation and fragment size (Castiglioni *et al.*, 2004). When applied to rRNA, band excision, re-amplification and sequencing of the products can be time and labor intensive, yet necessary when utilizing this method for species identification (Castiglioni *et al.*, 2004).

Filamentous with Heterocysts

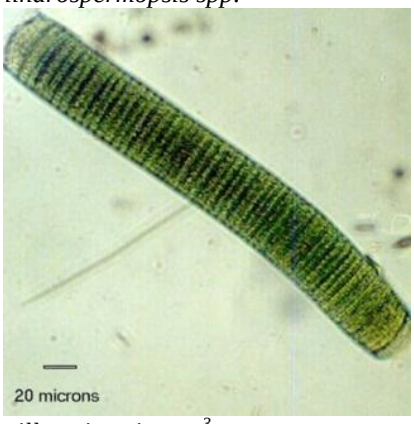


Cylindrospermopsis spp.[1]

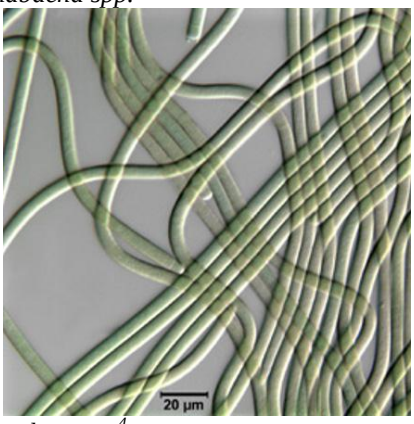


Anabaena spp.[2]

Filamentous without Heterocysts

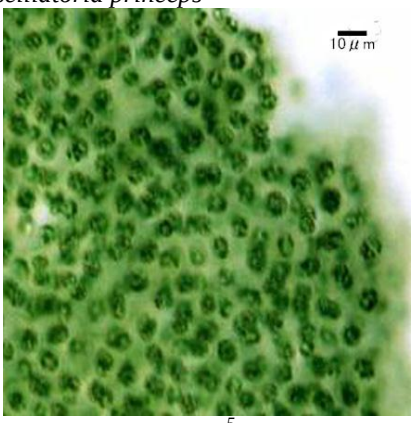


Oscillatoria princeps[3]

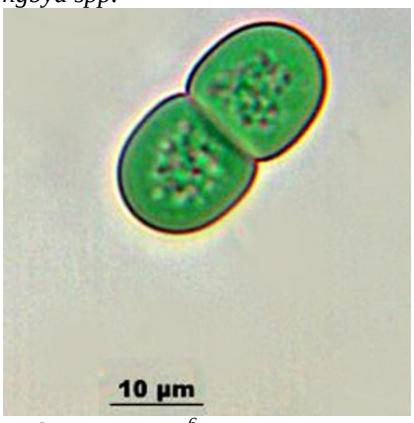


Lyngbya spp.[4]

Unicellular



Microcystis aeruginosa[5]



Synechococcus spp.[6]

1 http://www.glerl.noaa.gov/seagrant/GLWL/Algae/Cyanophyta/Images/Cylindrospermopsis.JPG
2 http://www.espacial.org/images/jpg2/anabaena_sperica.jpg
3 http://www-cyanosite.bio.purdue.edu/images/lgimages/PRINCEP1.JPG
4 http://silicasecchidisk.conncoll.edu/Pics/Other%20Algae/Blue_Green%20jpegs/Lyngbya5.jpg
5 http://www.icb.ufmg.br/~rmpc/img_t3.jpg
6 http://cfb.unh.edu/phycokey/Choices/Cyanobacteria/cyano_unicells/SYNECHOCOCCUS/Synechococcus_02_500x271_dr-ralf-wagner_de_blaualgen.jpg

Figure 1. Photomicrograph images representing the three major cyanobacteria morphologies. (after Paerl, 2008)

Oligonucleotide microarrays have been used in molecular research to study soil microbial diversity and are appropriate for broader environmental surveys (Small *et al.*, 2001). Rudi *et al.* (1998) confirmed PCR for use in quantifying cyanobacteria, and later developed probes which were used to make a microarray for genus-level identification and relative abundance calculations of cyanobacteria (Rudi *et al.*, 2000). Castiglioni *et al.* (2004) have also applied microarrays to cyanobacteria identification by using the ligation detection reaction. They designed a discriminating probe with a 5' fluorescent tag for each of 19 cyanobacterial groups. A common probe was also designed and group-specific 3' complementary zip (czip) codes were attached. Only complete matches of both probes would ligate in amplification, and the czip codes were then hybridized to the specific zip codes on the microarray. Fluorescence would occur when hybridization bound the zip code to the czip code. The microarray developed was "universal," in that the combination of zip codes could be applied to any probe pair.

Amplifying partial sections of the 16S rRNA using real-time PCR can be an efficient way to identify cyanobacteria. Using available cyanobacterial rRNA gene sequences, Nubel *et al.* (1997) developed primers for PCR (see Table 2). CYA359F and CYA781R were used and matched almost all of the published cyanobacteria sequences as of 1997. Many of the differences that were seen were attributed to sequencing errors. CYA106F was successfully used with CYA781R to generate PCR products despite one to three mismatches from the 16S genes from some strains of cyanobacteria. This primer pair amplified fragments that generally spanned two variable regions, V2 and V3, of the SSU ribosomal gene (Figure 2).

Table 2. PCR primers developed by Nubel *et al.* (1997) to identify cyanobacteria.

Universal Cyanobacteria Primers		
Primer	Sequence	Location in *E. coli* 16S rDNA
CYA 106F	CGGACGGGTGAGTAACGCGTGA	106-127
CYA359F	GGGGAATYTTCCGCAATGGG	359-378
CYA781R(a)	GACTACTGGGGTATCTAATCCCAT T	781-805
CYA781R(b)	GACTACAGGGGTATCTAATCCCTT T	781-805

All primers used have sequences that match other prokaryotic 16S rRNA sequences but when used in combination these mismatches are minimized (Nubel *et al.*, 1997). While it has been established that these primers are not necessarily universal, they seem to match most published sequences of cyanobacteria, as well as many published cyanelle and plastid sequences (Nubel *et al.*, 1997).

The emergent field of metagenomics has developed as a way to survey microbial populations and to display species diversity in uncultured samples. Metagenomic techniques have enabled previously unculturable microbial populations to be sequenced and identified. This has resulted in an increase in the known diversity of bacterial species (Hugenholtz *et al.*, 1998; Eisen, 2007). Using genomic DNA from lakes can help assess water quality, especially for drinking water reservoirs and recreational activities. Microbe populations can be quick indicators of changing water quality given their fast generation time (Devereux *et al.*, 2006). Metagenomic tools like quantitative PCR have been proposed for use to monitor water quality by comparing relative abundance of the bacterial community over time (Marshall *et al.*, 2008). Environmental shotgun

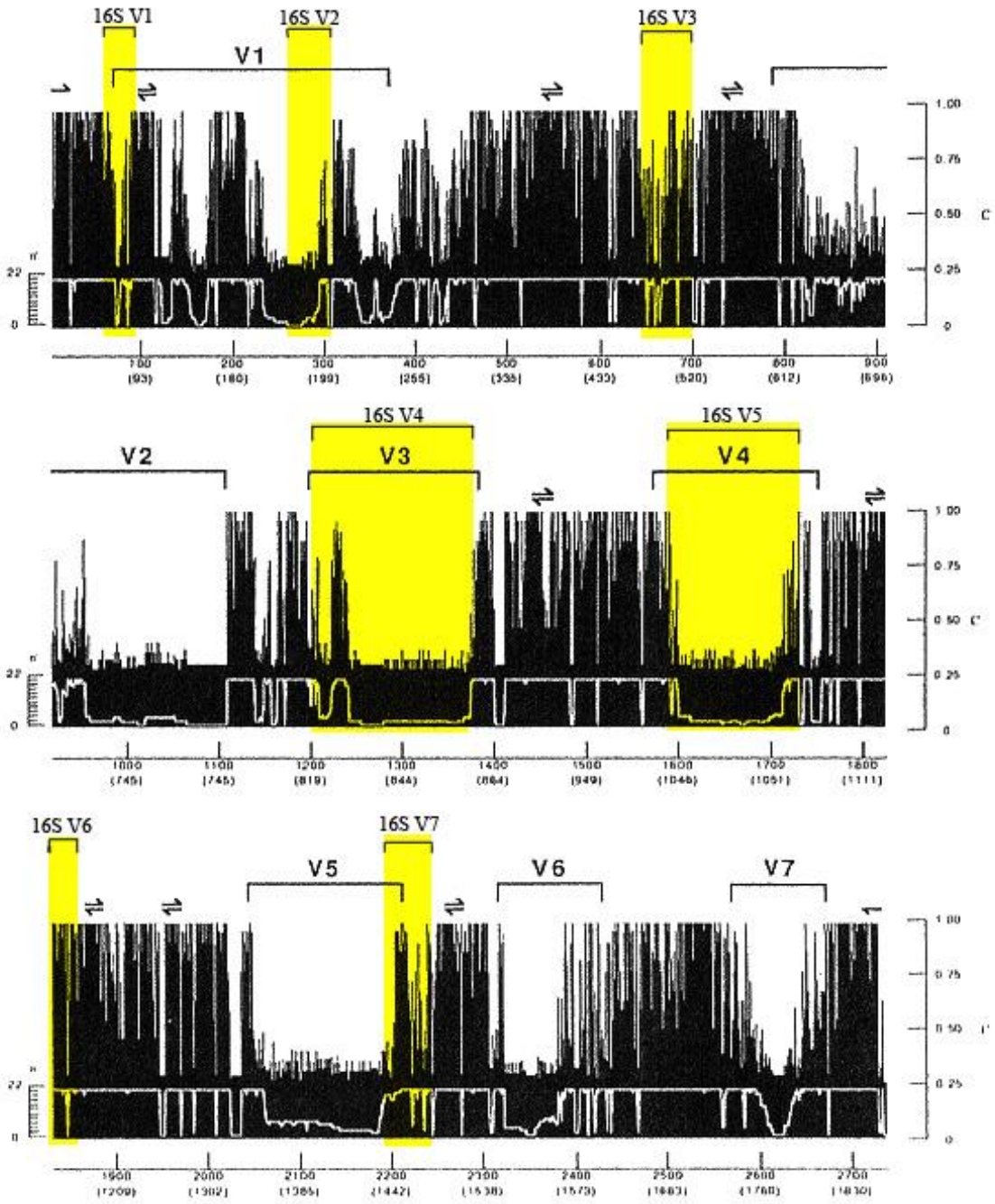


Figure 2. Variable regions of the 16S rDNA overlain on sequence conservation histogram of the 18S eukaryotic rDNA. (after Sogin and Gunderson, 1987; Lane, D.J., 1991)

sequencing provides random and broad samplings of a community (Eisen, 2007). Tyson *et al.* (2004) used shotgun sequencing of genomic DNA to reconstruct near complete genomes of the most common species in a bacterial community. More abundant organisms will have more copies of their genome present in a shotgun sequence, making a more complete picture of the genome possible.

Cyanobacteria are common in many of North Carolina's reservoirs (Touchette *et al.*, 2007). The specific objective of this study was to develop and test primers for real-time PCR analysis of cyanobacteria. Primers were developed using an existing set of sequence libraries from the variable regions of the 16S rDNA for specific operational taxonomic units (OTUs) and then validated against existing clones and previously identified pure cultures. Environmental samples from High Point City Lake were then analyzed as a practical test to assess cyanobacterial abundance and growth patterns. The expectation was that multiple cyanobacterial taxa would be present and that abundance of specific taxa would change as environmental parameters changed. For example, highest abundance would be expected during summer to early fall when water temperatures are warmest.

CHAPTER II

METHODS

Prior Generation of Sequence Libraries

Cyanobacteria sequence libraries were generated from water samples collected in 6 reservoirs by staff of the Center for Applied Aquatic Ecology at North Carolina State University and UNCG. Samples were collected in winter, summer, and fall from summer 2004 through winter 2006. Generally, 100ml of surface water was drawn through GFF filters and the filter was placed in cetyl trimethyl ammonium bromide (CTAB) buffer and stored at room temperature. The samples used to generate the sequences in the libraries (Table 3) were selected for high concentration of cyanobacteria, determined by measuring phycocyanin and by direct observation.

Sample libraries were generated in the following manner. DNA was extracted and purified from samples using a protocol developed by Doyle and Doyle (1987), as modified by Stewart and Via (1993) and Schaefer (1997). After purification, the sample DNA was resuspended in 20µl sterile ddH_2O and was quantified on a Nanodrop Spectrophotometer. PCR primers 106F and equimolar amounts of 781Ra and 781Rb (Table 2), developed by Nubel (1997), were used to amplify small subunit rRNA genes. PCR products were confirmed by gel electrophoresis. After confirmation, a PCR cleanup kit was used to purify the reaction mixture. The PCR product was cloned into Top-10 *E.*

coli cells using Invitrogen's TOPO TA Cloning Kit for Sequencing.

Cells were grown overnight on a spread plate of LB agar containing ampicillin. Individual colonies were picked and incubated overnight in 5ml LB + amp broth at 37° C. Cultures were then centrifuged for 20 minutes at 4000 RPM in a Beckman Coulter J2-HC centrifuge with a JS-4.3 rotor, and Qiagen QIAprep Spin Miniprep Kits were used to purify the plasmid clones. After confirming insert presence by gel electrophoresis, plasmid inserts were sequenced on an ABI MegaBACE 1000 using GE Healthcare DYEnamic ET Dye Terminator Kit.

Library Analysis and Primer Development

This study began with raw data sequence libraries from the sequencing runs (Table 3). Sequences were aligned and visually checked against the raw electropherogram output from the MegaBACE Sequence Analyzer software for erroneous base calls. Aligned sequences were queried using Basic Local Alignment Search Tool, BLASTn (Altschul *et al.*, 1997) to remove sequences that lacked identity with cyanobacterial ribosomal gene sequences. Sequences were then grouped into OTUs based on ≥0.975% similarity.

During OTU reanalysis, several OTU groups were found to be 97.5% similar to each other. These similar sequences were combined to form a single OTU. Conversely, some large OTUs contained sequences that, while within the 97.5% similarity criterion for a some sequences in the OTU, were still less than 97.5% similar to other members.

Table 3. Genomic DNA sources used to establish 16S rDNA library.

Lake	Sample ID	Date	# of Sequence Runs	Total # of Clones
Falls Lake	FL2-2	8/17/2006	4	155
High Point City Lake	HP-1	8/24/2006	4	187
Tuckertown Reservoir	TT-1	8/29/2006	4	138
Lake Wheeler	LW-1	8/2/2006	4	140
Jordan Lake	JD-1	8/28/2006	3	123
Badin Lake	BL-2	8/29/2006	3	143

In two cases (OTU4, OTU5) the divisions of these subgroups were sufficient to redefine them by separating them into two OTUs (OTU4a OTU4b, OTU5a, OTU5b). After establishing OTUs, all consensus sequences were compared to Genbank sequences (BLASTn, Altschul *et al.*, 1997), to establish identities.

PCR Primers were designed to consensus sequences of the OTUs using visual inspection of aligned sequences of each OTU. Primers were designed to have annealing temperatures between 60°-66° C, and to have between 44%-66% G/C content. Many clone sequences from the libraries did not include complete V2 and V3 regions of the SSU rDNA (Figure 2). Therefore, rather than develop primer pairs, forward primers for unique OTUs were paired with the CYA781R reverse primer. For those OTUs that were less variable, forward and reverse primers were designed to work together to develop OTU-specific amplification. In all cases, forward and reverse primers were selected to have similar annealing temperatures.

Primer testing and validation were conducted largely *in silico.* After potential primer pairs were identified, the primer sequences were BLASTed using local blast

(Altschul *et al.*, 1997) against the clone libraries to confirm specificity within the clone library. A nucleotide BLAST (Altschul *et al.*, 1997) was also performed to confirm that primers matched published cyanobacteria sequences. Primers generated in this study are listed in Appendix A.

Primers were validated against the original genomic DNA and clonal source DNA. First, the PCR was run using primers and the genomic DNA from the original field sample where the clone was isolated to confirm that they could amplify source material. Primers were then validated against dilutions of the DNA purified from the target clone. The dilution of target clone DNA also served as standards in subsequent qPCR runs. Applied Biosystems™ StepOne™ Real-time PCR System with SYBR® Green PCR Master Mix was used to perform primer validation and amplification. Reaction mixtures (Table 4) were selected based on the recommendations of the manufacturer and placed into 48-well plates. The real-time PCR reaction consisted of a holding stage for 10 minutes at 95ºC, followed by 35 cycles of 15 seconds at 95ºC, 30 seconds at 60ºC, one minute at 72ºC, and a 15-second data collection at 80ºC, followed by a melt curve analysis beginning at 95ºC, dropping to 60ºC and increasing at 0.3ºC every second until it reaches 95ºC again. After primers successfully amplified the target clonal DNA, a serial dilution for each OTU standard was made for use in later PCR runs.

Table 4. SYBR ® Green Master Mix reaction mixture.

PCR Reagents	Volumes
AB Power SYBR® Green Master Mix	10 µl
PCR Forward Primer (10 µM)	1 µl
PCR Reverse Primer (10 µM)	1 µl
Template (<100 ng)	1 µl
dH2O	7 µl
Total	20 µl

Eighteen genomic DNA samples were collected between December 2007 and December 2008 from High Point City Lake. Integrated surface-to-bottom samples were collected in 100ml aliquots, passed through GFF filters and stored in CTAB buffer at room temperature prior to DNA extraction. Samples were assayed for each OTU in 48-well reaction plates. Each plate contained 3 negative controls for DNA, 2 replicates for each sample, and 3 replicates of three serial dilutions. Generally, 3 replicates are accepted as an appropriate number in real-time PCR. Preliminary tests of the PCR methods indicated that our replicates were consistent and equal. With this in mind, 2 replicates were used on each plate which allowed simultaneous testing for each sample on one testing plate. If results on replicates were questionable, the reactions were re-run. Serial dilutions were of the original clonal standards. The 10% dilution series was selected based on the amount of DNA present in each original clone to match DNA concentration in samples.

Of 96 total OTUs identified in this study, 30 were selected for real-time PCR assessment in field samples from City Lake. These OTUs were selected for several reasons. First, the designed primers selected appeared to be OTU-specific and robust.

Second, some selected OTUs were similar to Genbank sequences of known potential toxin producing cyanobacteria. Third, the selected OTUs were some of the most abundant in the clone library, indicating the most relevance to NC reservoirs. Of the 30 OTUs assayed, 3 were removed after continued amplification in the negative controls and because a melt curve analysis suggested that amplification in these samples was non-specific. Two OTUs had no amplification, suggesting that they were not present in the City Lake samples.

CHAPTER III

RESULTS

Upon OTU assignment, sequences were queried to Genbank (BLASTn, Altschul *et al.*, 1997) to determine likely identities (Table 5). Genbank accessions with similarities higher than 97.5% are generally considered to be within the same species. Only 3 of the 100 OTUs were identified to a species level with confidence (Sequence similarity ≥97.5% to a named species in Genbank). 17 OTUs had similarity values ≥97.5% to an entry in Genbank, but could only be identified to the genus level or were sequences that had been found previously but were not identified to any known genus or species. 80 OTUs had low sequence similarities, again apparently representing unknown taxa. Cyanobacteria OTU richness (number of different OTUs detected) was highest during the summer to fall months, peaking at 25 OTUs in late July (Figure 3). OTU richness was generally below 15 OTUs during other times of the year. Of the total cyanobacteria OTUs identified in the 18 samples, 11 OTUs were found in all samples. The abundance of targeted cyanobacterial DNA was highest between 18-Jun-08 to 19-Oct-08 (Figure 4). Lower values were found during other times of the year although there were smaller peaks in March and May. An early June sample had very low abundance of targeted cyanobacteria DNA.

Table 5. List of operational taxonomic units (OTUs) found in this study with best GenBank match (accession #, description, source location, and % similarity) to the consensus OTU sequence. Taxonomic identification is given where possible. Similarity values ≥ 98% are noted in bold. Note that OTUs 13_70, 27, and 36 are likely eukaryotic chloroplast 16S ribosomal genes. OTUs surveyed in this study are outlined in black.

OTU	Best Genbank Match	Source Location of Genbank Match	% similarity	ID	Accession
1	unidentified cyanobacterium clone LD7	Lake Loosdrecht, Netherlands	96%	*Prochlorothrix hollandica*	AJ007864.1
2	Cylindrospermopsis raciborskii strain Florida F	Florida, USA	**100%**	*Cylindrospermopsis raciborskii*	AF516745.1
3	Synechococcus sp. LBG2	Lake Biwa, Japan	**99%**	Synechococcus sp.	AF330249.1
4a_74	Uncultured bacterium clone TH_d324	Lake Taihu, China	**99%**		EU273090.1
4b	Uncultured bacterium clone LK15m-37-16S	Lake Kinneret, Israel	**99%**		GU131246.1
5a	Uncultured bacterium clone LK20m-8A-16S 16S	Lake Kinneret, Israel	98%		GU131247.1
5b	Uncultured bacterium clone LK20m-8A-16S 16S	Lake Kinneret, Israel	99%		GU131247.1
6	Uncultured cyanobacterium clone H1w-5	Salar de Huasco, Chile	92%		EF633015.1
7_96	Uncultured Plectonema sp. clone 2K89	Ground water, Israel	97%		GU074273.1
8_57	Aphanizomenon issatschenkoi LMECYA 190	Maranhão Reservoir, Portugal	**100%**	*Aphanizomenon issatschenkoi*	EU078536.1
9	Uncultured bacterium clone MFBC7A05	Tucurui Reservoir, Brazil	95%	*Synechococcus* sp.?	EU592776.1
10	Uncultured cyanobacterium clone SHWN_night2_16S_697	estuary, Georgia, USA	93%		FJ745161.1
11	Uncultured cyanobacterium clone OO.P3.LT.46.ab1	Monterrey Bay, CA, USA	92%		HQ821728.1

OTU	Best Genbank Match	Source Location of Genbank Match	% similarity	ID	Accession
12	Anabaena sp. XPORK15F	Gulf of Finland	96%	*Anabaena* sp.	EF568905.1
13_70	Uncultured eukaryote clone ML-9-3 plastid	Lake Taihu, China	**99%**		DQ166477.1
14	Uncultured cyanobacterium clone ADK-SGh02-76	Adirondack Lake, NY, USA	97%		EF520521.1
15	Uncultured bacterium clone EDP-26	pond, Dongshan Island, China	91%		EU283208.1
16	Uncultured cyanobacterium clone ADK-SGh02-76	Adirondack Lake, NY, USA	94%		EF520521.1
17	Uncultured cyanobacterium clone ND2_CYA_1_8	Lake Doirani, Greece	97%	*Psuedanabaena sp.?*	FJ204854.1
18	Spirulina laxissima strain SAG 256.80	Laka Nakuru, Kenya	95%	*Spirulina* sp.?	DQ393278.1
19	unidentified cyanobacterium clone LD7	Lake Loosdrecht, Netherlands	92%		AJ007864.1
20	Uncultured Cyanobacterium sp. clone 3-73_EH_284_Apr_2010	Erhai Lake, China	95%		HQ724800.1
21	Uncultured bacterium clone H_10	Dry Valley, Antarctica	92%		FJ490330.1
22_51	Cylindrospermopsis raciborskii (Raphidiopsis sp. D9)	Billing reservoir, Brazil	95%	*Cylindrospermopsis* sp.?	EU552070.1
23	Uncultured cyanobacterium clone ND2_CYA_1_8	Lake Doirani, Greece	**98%**		FJ204854.1
24	Uncultured bacterium clone H_10	Dry Valley, Antarctica	93%		FJ490330.1
25	Uncultured cyanobacterium clone ND2_CYA_4_32	Lake Doirani, Greece	91%		FJ204843.1
26	Anabaena spiroides LMECYA 161 C20	Agolada de Baixo Reservoir, Portugal	97%	*Anabaena* sp.	EU078524.1
27	Heterosigma akashiwo strain CCMP 452 plastid	Long Island Sound, USA	89%		EU168191.1

OTU	Best Genbank Match	Source Location of Genbank Match	% similarity	ID	Accession
28	Anabaena mendotae 04-45	Svet fishpond, Czech Republic	94%		FM242084.1
29	Uncultured cyanobacterium clone ADK-SGh02-76	Adirondack Lake, NY, USA	95%		EF520521.1
30	Uncultured cyanobacterium clone ADK-SGh02-76	Adirondack Lake, NY, USA	96%		EF520521.1
31	Cylindrospermopsis raciborskii CS-511	McKinlay farm dam, QLD, Australia	94%		EU552068.1
32	Uncultured cyanobacterium clone 16L6	Georgia, USA	92%		EU409863.1
33	Uncultured bacterium clone TH_d324	Lake Taihu, China	99%		EU273090.1
34	Nostoc sp. 8941	New Zealand	95%		AY742448.1
35	Uncultured bacterium HOClCi65	drinking water simulator, USA	**98%**		AY328614.1
36	Uncultured phototrophic eukaryote clone ND2_CYA_1_22 plastid	Lake Doirani, Greece	95%		FJ204841.1
37	Uncultured cyanobacterium clone SHWN_night2_16S_697	estuary, Georgia, USA	93%		FJ745161.1
38	unidentified cyanobacterium clone LD7	Lake Loosdrecht, Netherlands	94%		AJ007864.1
39	Cylindrospermopsis raciborskii (Raphidiopsis sp. D9)	Billing reservoir, Brazil	95%		EU552070.1
40	Uncultured bacterium clone CBM01H09	Chesapeake Bay, MD, USA	95%		EF395686.1
41	Cylindrospermopsis raciborskii (Raphidiopsis sp. D9)	Billing reservoir, Brazil	96%		EU552070.1
42	Uncultured bacterium clone MFBC5F11	Tucurui reservoir, Brazil	**98%**		EU592798.1
43	Uncultured bacterium clone CHPA.0912.21	Lake Chaohu, China	92%		HQ904418.1

OTU	Best Genbank Match	Source Location of Genbank Match	% similarity	ID	Accession
44	Uncultured cyanobacterium clone OO.P2.OT.96.ab1	Monterrey Bay, CA, USA	88%		HQ821686.1
45	Uncultured cyanobacterium clone ADK-SGh02-76	Adirondack Lake, NY, USA	97%		EF520521.1
46	Uncultured cyanobacterium clone NK2_CYA_2_1	Lake Kastoria, Greece	92%		FJ204874.1
47	Anabaena sp. strain A277	River Perniönjoki, Finland	94%		AJ133160.1
48	Uncultured cyanobacterium clone TH_f27	Lake Taihu, China	92%		EU980182.1
49	Uncultured bacterium clone DP10.2.55	Lake water, China	**98%**		FJ612358.1
50	Uncultured bacterium HOClCi65	drinking water simulator, USA	95%		AY328614.1
52	Uncultured bacterium clone PVP-121	pond, Dongshan Island, China	92%		EU283259.1
53	Uncultured cyanobacterium clone NV1_CYA_1_12	Lake Volvi, Greece	95%		FJ204890.1
54	Uncultured bacterium clone MFBC9A02	Tucurui reservoir, Brazil	92%		EU592777.1
55	Uncultured bacterium clone KCLunmb_25_16	spring sediment, Taiwan	92%		FJ638592.1
56	Nostoc sp. 8941	New Zealand	96%		AY742448.1
58	Aphanizomenon issatschenkoi LMECYA 166	Vale Michões Reservoir, Portugal	96%	*Aphanizomenon* sp.	EU078535.1
59	Cylindrospermopsis raciborskii (Raphidiopsis sp. D9)	Billing reservoir, Brazil	97%		EU552070.1
60	Unidentified cyanobacterium clone LD25	Lake Loosdrecht, Netherlands	95%		AJ006286.1
61	Uncultured cyanobacterium clone LW9m-1-3	Lake Michigan, WI, USA	93%		EU641407.1

OTU	Best Genbank Match	Source Location of Genbank Match	% similarity	ID	Accession
62	Uncultured cyanobacterium clone H6w-73	Salar de Huasco, Chile	94%		EF632984.1
63	Uncultured cyanobacterium clone B97	South China Sea, China	94%		FJ999606.1
64	Uncultured bacterium clone MFBC10H09	Tucurui reservoir, Brazil	95%		EU592816.1
65	Uncultured cyanobacterium clone ADK-SGh02-76	Adirondack Lake, NY, USA	97%		EF520521.1
66	Uncultured bacterium clone MFBC2C08	Tucurui reservoir, Brazil	**99%**		EU592789.1
67	Uncultured cyanobacterium clone LK1mC-7	Lake Kinneret, Israel	94%		DQ158169.1
68	Uncultured bacterium clone TH_d324	Lake Taihu, China	93%		EU273090.1
69	Uncultured cyanobacterium clone H4s-61	Salar de Huasco, Chile	93%		EF632962.1
71	Uncultured Skeletonema sp. clone 2K19	Groundwater, Isreal	94%		GU074215.1
72	Uncultured cyanobacterium clone TH_h52	Lake Taihu, China	95%		EU980295.1
73	Unidentified cryptomonad OM283	Cape Hatteras, NC, USA	94%		U70724.1
75	Uncultured bacterium clone P4O-58	Puma Yumco Lake, Tibet, China	94%		EU375434.1
76	Uncultured bacterium clone nbw345e08c1	human skin bacterium	97%	*Synechococcus* sp.?	GQ091396.1
77	Uncultured cyanobacterium clone ADK-SGh02-76	Adirondack Lake, NY, USA	97%		EF520521.1
78	unidentified cyanobacterium clone LD7	Lake Loosdrecht, Netherlands	94%		AJ007864.1
79	Anabaena sp. strain A277	River Perniönjoki, Finland	95%		AJ133160.1

OTU	Best Genbank Match	Source Location of Genbank Match	% similarity	ID	Accession
80	Uncultured bacterium clone CBM01C08	Chesapeake Bay, MD, USA	93%		EF395634.1
81	Uncultured bacterium clone CBM01C08	Chesapeake Bay, MD, USA	93%		EF395634.1
82	Uncultured Verrucomicrobiales clone LW18m-2-26	Lake Michigan, WI, USA	**98%**		EU642323.1
83	Uncultured Opitutae bacterium clone YL186	Yellowstone Lake, WY, USA	**98**		HM856547.1
84	Uncultured cyanobacterium clone Erie 24	Lake Erie, USA	94%		AY858017.1
85	Uncultured Antarctic cyanobacterium clone FreP30	Pond, McMurdo Shelf, Antarctica	95%		AY541558.1
86	Uncultured cyanobacterium clone NK2_CYA_2_1	Lake Kastoria, Greece	93%		FJ204874.1
87	Uncultured cyanobacterium clone LK1mC-3	Lake Kinneret, Israel	97%	*Synechococcus* sp.?	DQ158166.1
88	Uncultured Synechococcus sp. clone CB22A07	Chesapeake Bay, MD, USA	95%		EF471533.1
89	Uncultured bacterium clone Reef_M07	Caribbean Sea	95%		GU119187.1
90	Nodularia spumigena GSL023 e	Great Salt Lake, UT, USA	**98%**	*Nodularia spumigena*	FJ546713.1
81	unidentified cyanobacterium clone LD7	Lake Loosdrecht, Netherlands	91%		AJ007864.1
92	Uncultured cyanobacterium clone GR1G3	Green Lake, WI, USA	94%		FJ916323.1
93	Uncultured phototrophic eukaryote clone PRD18H11	Parker River, MA, USA	94%		AY948073.1
94	Uncultured cyanobacterium clone NK2_CYA_2_1	Lake Kastoria, Greece	91%		FJ204874.1
95	Uncultured cyanobacterium clone NV1_CYA_1_12	Lake Volvi, Greece	**99%**		FJ204890.1
97	Uncultured cyanobacterium clone LK1mC-7	Lake Kinneret, Israel	**98%**	*Plectonema* (*Leptolyngbya*) sp.	DQ158169.1

OTU	Best Genbank Match	Source Location of Genbank Match	% similarity	ID	Accession
98	Uncultured bacterium clone XYHBP.0912.47	Lake Xingyunhu, China	**98%**		HQ904577.1
99	Uncultured bacterium clone Gerber_L7-E3-T7	Lake Gerber, Spain	97%		FN297771.1
100	Uncultured bacterium clone IFBC1C11	Tucurui reservoir, Brazil	96%		EU592544.1

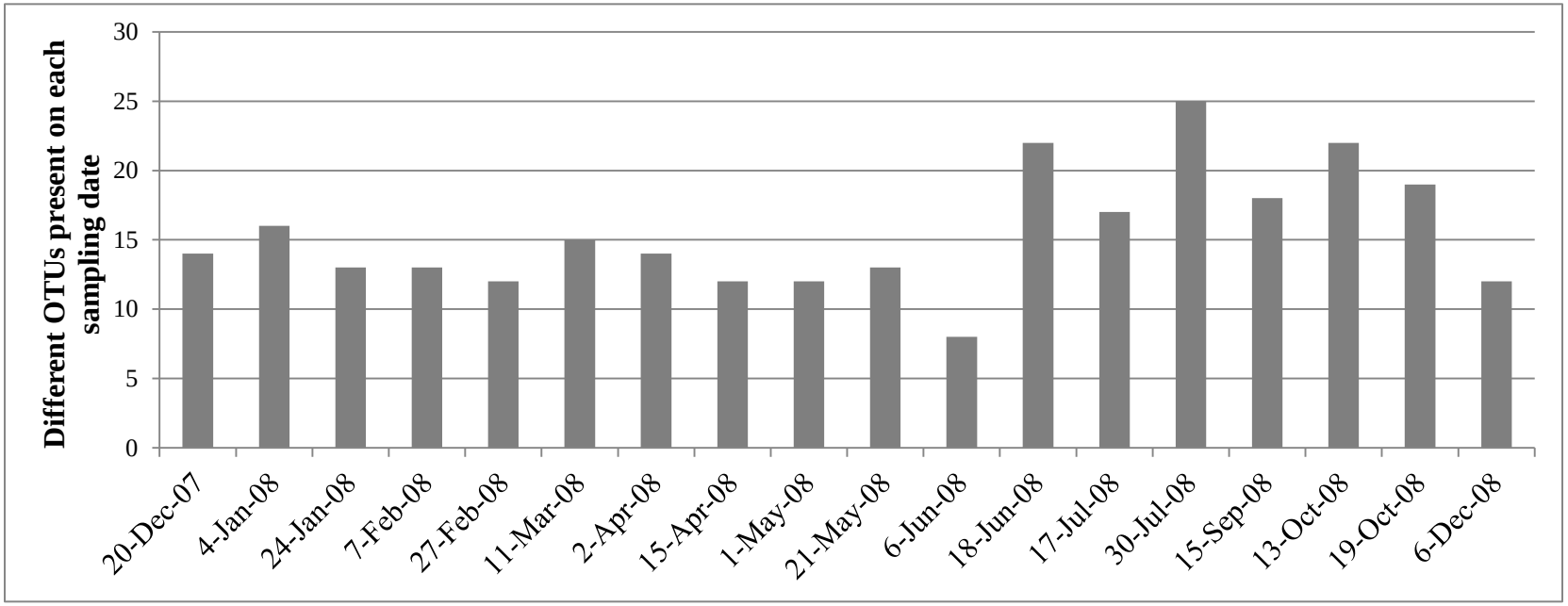


Figure 3. Total OTUs present on each sampling date.

The most common OTU present over the sampling period was OTU 11, followed closely by OTU 89, both of which were found throughout the year (Figure 4). OTU 67 was also abundant, but only on two sampling dates during the summer. The remaining OTUs made up a smaller portion of the total cell counts (Figures 4, 5).

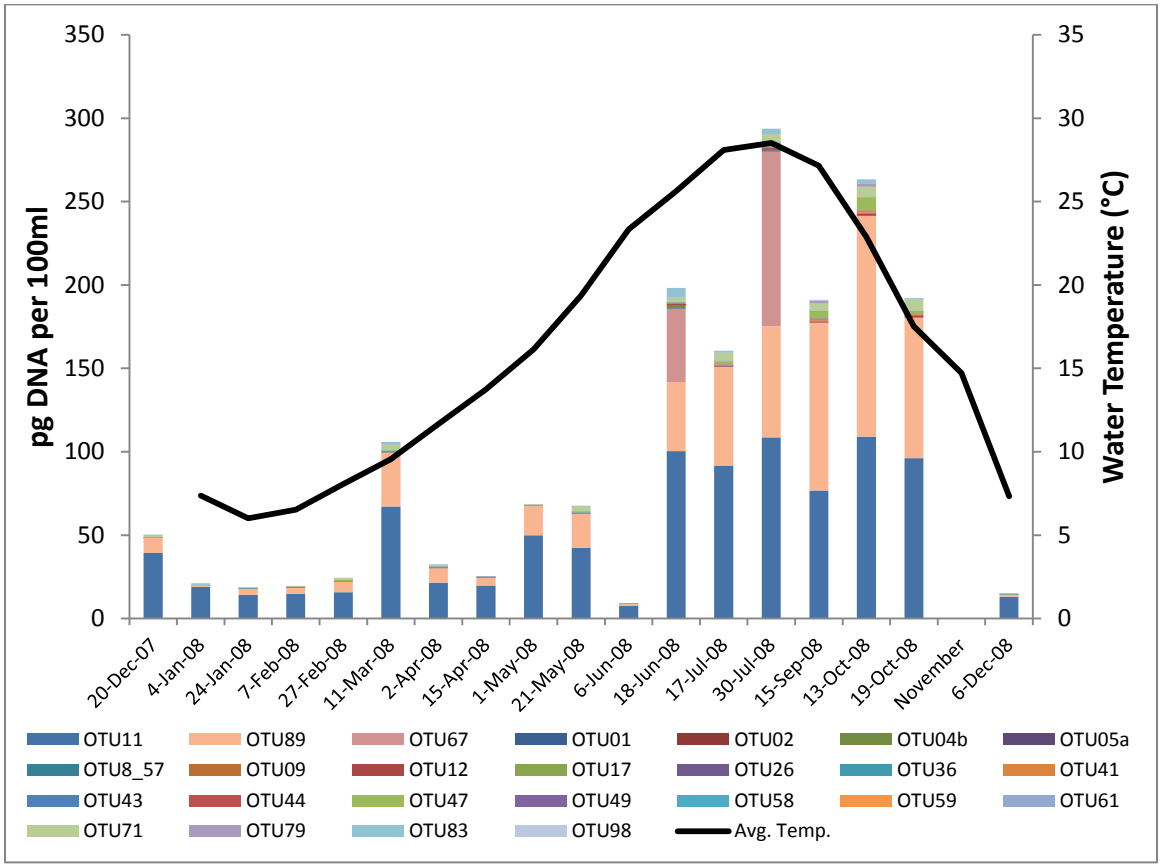


Figure 4. Cyanobacteria DNA grouped by OTU, overlain with average temperature

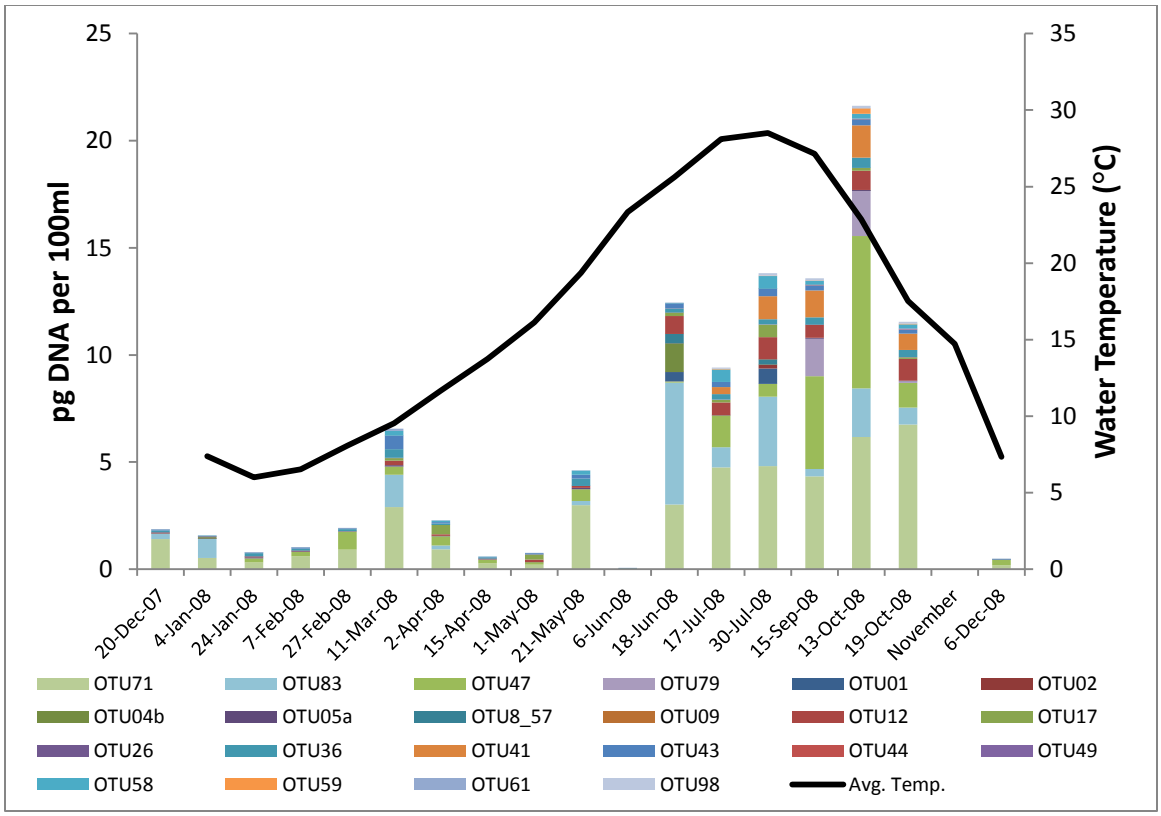


Figure 5. Cyanobacterial DNA grouped by OTU, with OTU11, OTU67 and OTU83 removed. Overlay of average temperature. Note that y-axis scale is 10-fold less than in Figure 4.

Environmental variables temperature, dissolved oxygen (DO), pH, turbidity (Turb), phycocyanin (Phyco), and chlorophyll a (CHLA) were also monitored as part of the Real-Time Remote Monitoring System (RTRM) operated by the NC State Center for Applied Aquatic Ecology (CAAE). They also conducted laboratory assays for suspended solids (SS), total kjeldahl nitrogen (TKN), total inorganic nitrogen (TIN), total nitrogen (TN), total phosphorus (TP), TN/TP ratio (TN:TP), ammonia (AMMO), and total organic carbon (TOC). Detailed results from the RTRMs can be found at the NCSU CAAE

website (http://www.ncsu.edu/wq), and relevant data are also plotted against OTU target abundance in Figures 6 and 7.

To determine if there were any significant relationships between total OTU DNA and the environmental variables, a Spearman Rank Order Correlation was calculated. Temperature showed a significant correlation with total OTU abundance (P=0.00321) as did DO (P=0.0497), and TKN (P=0.0173), but most parameters were not significant (P>0.05). Correlations for each OTU were also calculated. Many showed a correlation with temperature. None of the OTUs were significantly related to turbidity, suspended solids, total nitrogen or chlorophyll a (Appendix C).

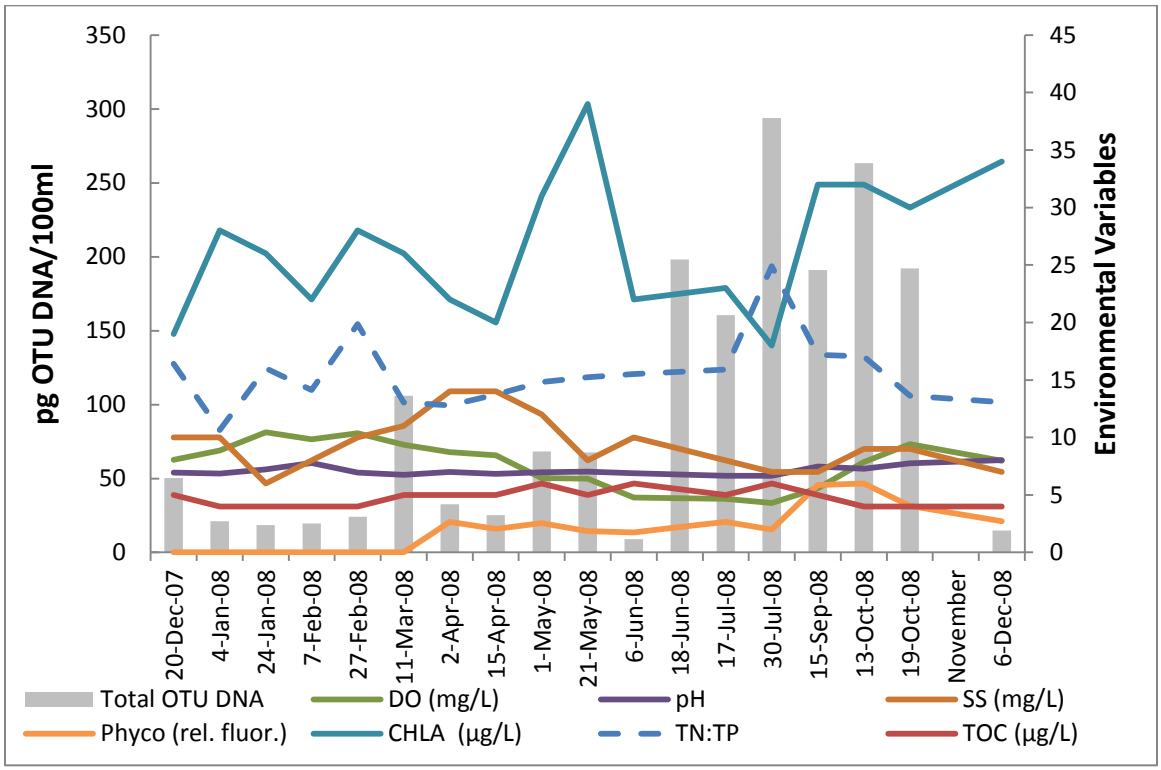


Figure 6. Target OTU DNA overlain with low measure environmental data points.

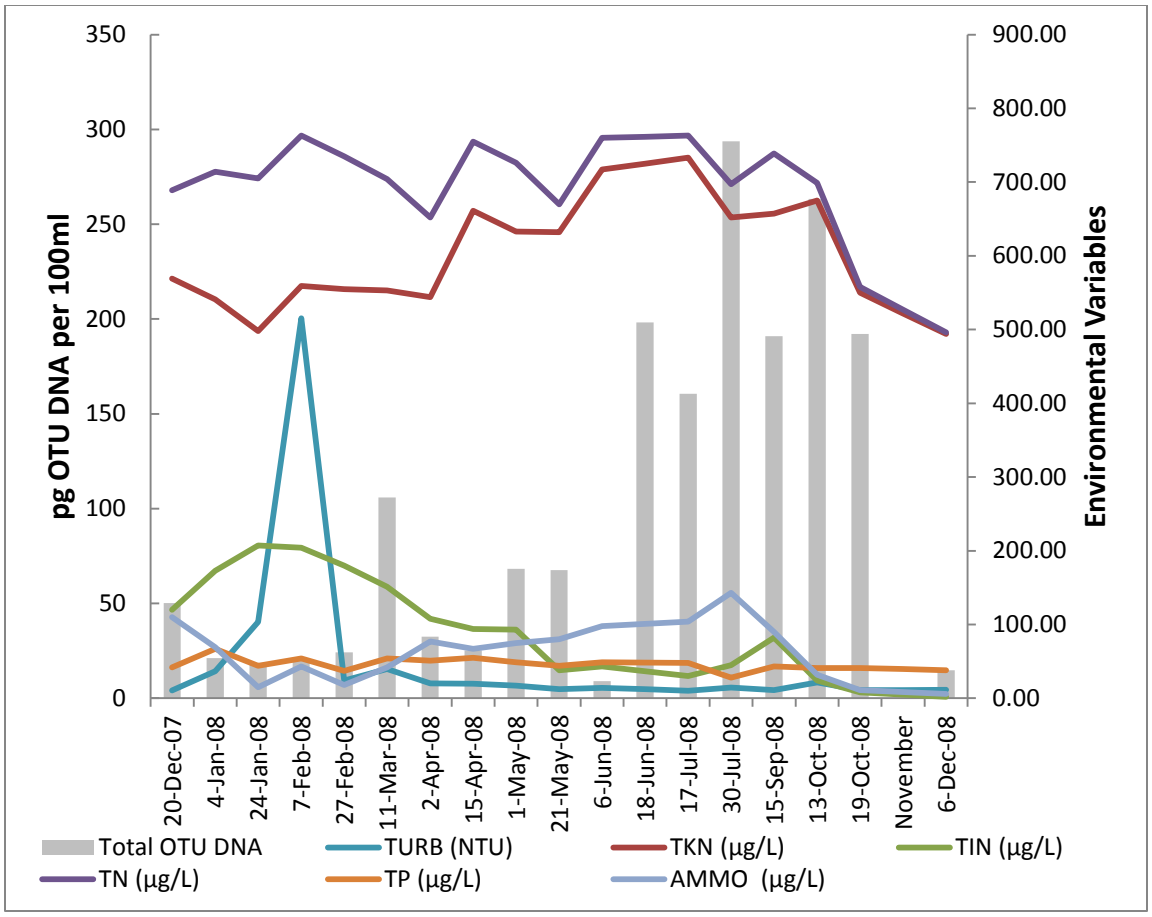


Figure 7. Target OTU DNA overlain with high measure environmental data points.

CHAPTER IV

DISCUSSION

In this study, cyanobacterial 16S rDNA genes were assayed using Real-time PCR to identify and quantify the number and relative abundance of cyanobacterial OTUs present in City Lake. Primers were developed and validated against clonal DNA derived from cyanobacterial communities found in six North Carolina drinking water supply reservoirs. Of 96 OTUs identified from the 883-sequence library, 30 OTUs were assayed against 18 genomic DNA samples from different dates from December 2007 thru December 2008. OTU presence was confirmed and DNA was quantified from standard concentrations.

Overall, cyanobacteria target DNA matched the expected seasonal patterns. Two sampling dates did have unusual DNA amounts. The sampling on 6-Jun-08was expected to have a high target DNA amount. It had an abnormally low count, however. This could be attributed to three potential factors. First, environmental factors were considered. Ahn *et al.*(2011) studied 14 environmental factors related to cyanobacterial density. They found that temperature was most strongly related when it was measured 2-4 weeks before a bloom. Temperature measurements before and after 6-Jun-08 match the expected temperatures for the time of year. Second, rainfall events can change many aspects of lacustrine chemistry. Oversaturation of nutrients, destratification of the water column, and reduction in in light intensity as a result of rain events plays an important

factor in the reorganization of the phytoplankton community (Moura *et al.*, 2011). Ahn *et al.* (2011) showed that rainfall events have strong effects on cyanobacteria density when the rainfall occurs 3 weeks prior to sampling. The only large rain event in the 3 weeks prior to sampling on 6-Jun-08 was one week before the sample. Third, sample DNA from 6-Jun-08 may have had an error in DNA extraction. Without rainfall events or changes to nutrients and other environmental factors, this is likely the reason for the departure from the expected target DNA amounts.

Another result different than expected was the total target DNA from 11-Mar-08. This sampling date was higher than the other winter and spring sampling dates. As mentioned above, rain events may cause a substantial increase of nutrients and turbidity causing a reduction in light intensity (Moura *et al.*, 2011). Turbidity measurements approximately 4 weeks prior to this sampling date are considerably higher than the other sampling dates. It has been suggested that cyanobacterial diversity can be affected by environmental factors up to 5 weeks before a bloom (Ahn *et al.*, 2011) and that increased turbidity may reduce overall phytoplankton biomass. However, Spearman Rank Order Correlation found no significant relationship between Total OTU DNA and Turbidity ($P=0.0665$).

The community of cyanobacteria in City Lake appears to be diverse. There were several OTUs that were present in the samples throughout the year. It is appropriate to note that of the 27 OTUs surveyed in City Lake, 3 appeared to dominate (Figure 8). OTU 11 and OTU 89 were present in highest concentrations on most sampling dates. OTU67, while only present on two sampling dates, had the third-highest DNA

concentration over the entire sampling period. The highest similarities from Genbank for these OTUs are less than 97.5% similar to any documented sequence (Table 5). The

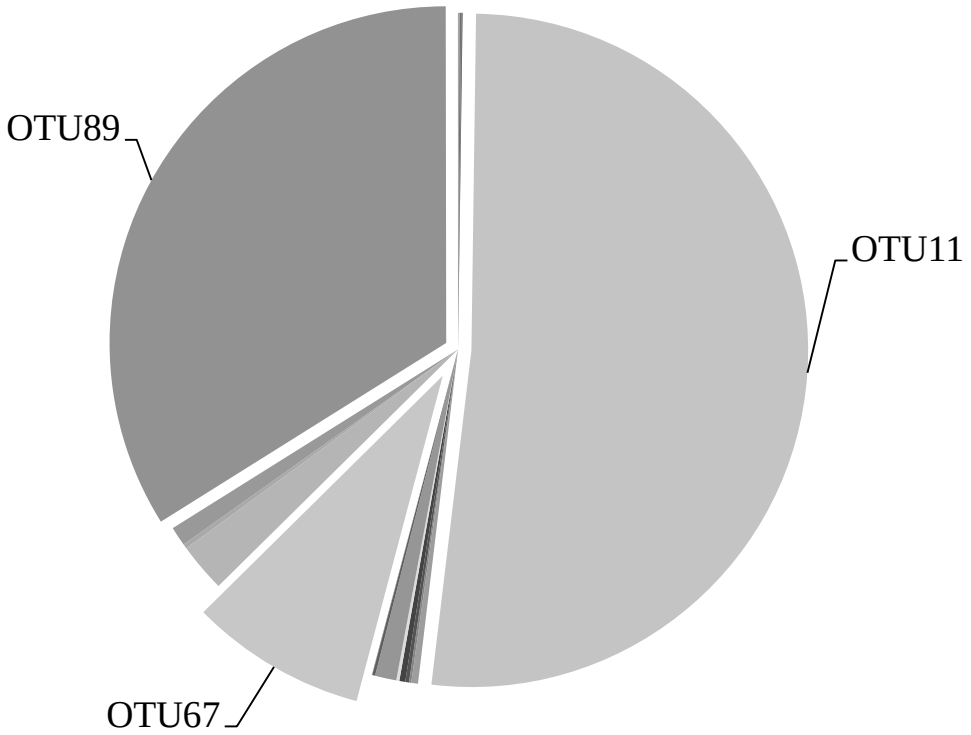


Figure 8. Proportion of total sampled DNA represented by the three dominant OTUs.

best matches are uncultured cyanobacterium and bacterium clones from locations around the world (Table 5). The high abundance of these three OTUs and the lack of species identification suggests that much more research needs to be done on cyanobacteria in order to establish identities of these OTUs.

Although the results of this study generally give expected results and the approach has been used in other studies (Schaefer, 1997; Marshall, 2002; Marshall *et al.*, 2008),

they do not provide a complete evaluation of the cyanobacterial community. When Nubel *et al.*(1997) designed their universal primers for cyanobacteria, there were only 174 16s rRNA sequences published. There is a distinct possibility that other genera of cyanobacteria have not been accurately assayed in this study simply because they do not match the CYA106F and CYA781R primers developed by Nubel *et al.* (1997). Additionally, alternate methods for DNA extraction might improve quality of genomic template (Arbeli and Fuentes, 2007; Yilmaz *et al.*, 2009).

This has been largely a methods development study. The methods used are generally accepted as appropriate for this task. One study developed a clone library in the same manner as this study, but rather than developing primers and amplifying fragments, sequences were compared to Genbank results to determine species diversity (Lymperopoulou *et al.*, 2011). Revetta *et al.* (2011) also used a similar approach. Novel cyanobacteria groups were identified in treated drinking water, suggesting that some cyanobacteria can withstand disinfection (Revetta *et al.*, 2011).

The OTUs identified from the sequence library had primers developed and tested. Consensus sequences for those OTUs were compared to Genbank results and identities for 97.5% similar sequences were found. Only three of the OTU library sequences matched (>97.5% similarity) published sequences of identified cyanobacteria (Table 5). Of those three, OTU02 (*Cylindrospermopsis raciborskii*) and OTU08_57 (*Aphanizomenon issatschenkoi)* were assayed in this study. OTU02 was present on four sampling dates and OTU08_57 was present on three sampling dates (Appendix B). Most of the OTUs matched unknown taxa of cyanobacteria. These unknown cyanobacterium

clones provide data useful in assessing community diversity. Dominant cyanobacteria taxa in North Carolina reservoirs include species of *Planktolyngbya, Aphanizomenon, Cylindrospermopsis, Anabaenopsis, Aphanocapsa, Oscillatoria, Anabaena,*and *Microcystis* (Glasgow and Burkholder, 2003). To further survey lakes using the methods in this study, published primers for other known cyanobacteria should be utilized or new primers should be generated. Insight into the structure of the cyanobacterial community would be increased if primers for more well-known, identified taxa were assayed in addition to the OTU primers.

Cyanobacteria are present in High Point City Lake year-round. Given the potential for blooms and toxin production as well as the cost of treating water that contains cyanobacteria, there is incentive to monitor and pro-actively manage cyanobacteria in the water supply. High Point City Lake contains destratification bubblers. These bubblers function to decrease anoxic conditions in the hypolimnion (Sahoo and Luketina, 2005) that can result in the release of benthic nutrients, notably phosphorous, which can lead to algae growth including cyanobacterial blooms. Decomposition of cyanobacteria can also exacerbate the anoxic conditions as well as release undesirable smell and taste compounds into the water (Sahoo and Luketina, 2005). These bubblers also have been shown to increase algal biodiversity (Burford and Davis, 2011) and decrease *Anabaena* concentrations (Castelletti *et al.*, 2010). However, broadly tolerant species such as *Cylindrospermopsis raciborskii* may thrive in destratified conditions (Burford and Davis, 2011).

Another method for reducing the concentration of cyanobacteria in City Lake may be applications of hydrogen peroxide at upstream wastewater treatment plants (Barrington and Ghadouani, 2008). This method produces results similar to UV light treatment and can be used to treat wastewater effluent for cyanobacteria before releasing it into the waterway (Barrington and Ghadouani, 2008). One bioremediation technique used successfully in Lake Taihu, China is an aquatic vegetation bed. These plantings support communities of bacteria and protozoa that help to remove cyanobacteria as well as the toxins they produce (Song *et al.*, 2009).

Cyanobacteria that produce toxins are present in High Point City Lake (Glasgow and Burkholder, 2003). The approach used in this study does not inform on cyanotoxins, just the organisms that produce them. To detect cyanotoxins at this time additional assays like ELISA must be used (Paulino *et al.*, 2008). Another approach to toxin detection is that reverse transcriptase quantitative PCR could be developed to assess expression of toxin genes. Nevertheless, the approach used here offers promise for improved assessment of cyanobacterial abundance in lake communities.

Future approaches with real-time PCR to assess cyanobacteria in a genomic DNA sample would be more useful to end-users if they estimated cell counts. Fogel *et al.* (1999), in a literature review, documented three cyanobacteria species that have 2 copies each of the SSU rDNA. Double-stranded DNA has a mass of 607.4 g/mol ("Ambion's Appendix - DNA and RNA Molecular Weights and Conversions," 2011) and the average of 24 surveyed cyanobacteria genomes contains 6149 ±1915 kilobases (Fogel *et al.*, 1999). Using these values, it can be estimated that there are 6.2 ± 1.9 fg DNA/cell. Cell

counts estimation will be much improved when knowledge of conversion factors for a broader range of representative species are determined.

This study could also be improved by assaying the rest of the primers designed for the other OTUs in the clone library and by expanding the number of targeted clones. While this study has shown that real-time PCR can be used to monitor cyanobacteria in southeastern reservoirs, more OTUs need to be examined to accurately reflect the total cyanobacterial diversity across a broad range of lakes. Some rudimentary results have been gathered but the scope of this information does not lead to great insight into the diversity of cyanobacteria in reservoirs. To improve these results, assays of the cyanobacterial abundance in more lakes should be done. Another way to increase the insight of this study into the cyanobacterial community would be to include more sampling dates. Short-term events or blooms may have been missed due to the sampling schedule. Primers have been and continue to be developed to cyanobacteria known to cause harmful algal blooms. These primers should be incorporated into future studies.

The success of this study suggests that real-time quantitative PCR is a method that has great potential for monitoring cyanobacteria diversity in aquatic ecosystems. More extensive studies across broad geographic ranges will be needed to confirm the widespread utility of this approach.

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APPENDIX A:

PCR PRIMERS

PCR Primers generated during this study. A: Primer sets with a forward primer to a single OTU paired with equimolar amounts of reverse primers CYA781R(a) and CYA781R(b). B: Forward primers designed to multiple OTUs followed by the unique OTU reverse primers.

A:

OTU	Primer Sequence	Length	% GC	Tm	Amplicon
OTU02	GGTGAAAGATTTATCGCCTGGAGATGA	27	44.4	63.45	614
OTU04(b)-33	CCTTAGGAGGAGGATACAGCT	21	52.4	59.82	668
OTU05a	GGATGTATCCACCTTAGGAAGAGCT	26	46.2	63.22	607
OTU11F (a)	CGGAAACGACTGCTAATACCTTATATG	27	40.7	61.93	647
OTU12	GGTAGTGTAAGAGACAACCAAGG	23	47.8	60.65	565
OTU13-70F	GGATTTATCTACCTTAGGAAGAGCTC	26	42.3	61.65	606
OTU15F (a)	CCTCTGCCTGAAGAGAAGCT	20	55	59.35	584
OTU18F (a)	GGTTGGGACAACCATTGGAAAC	22	50	60.25	663
OTU23 (b)	CCTACAGACTCGGGACACAG	20	60	61.4	669
OTU25F (a)	CCTCTAGGAAAGGGATAACAATCG	24	45.8	61.01	669
OTU26	CCCTCAGGTCGGGGACAACCA	21	66.7	65.68	669
OTU27F (b)	CCTTTAGGAAAGGGACACAATTGGAAAC	28	35.7	60.73	669
OTU32F	CCCTAGGGTGAAAGATTAATTGCCA	25	44	61.34	622
OTU36	GGATGACAGCCCTTGGGTTGTAAA	24	50	62.72	405
OTU38	CCTAGTCGGGGACAACAGTTGGAA	24	54.2	64.43	668
OTU39F	GCTCAGTCGGGATAACAGTTGAA	23	47.8	60.65	670
OTU40F	CGGGATACCGCCTGAGAATGA	21	57.1	61.78	607
OTU41F (b)	GAGATCTGCTCCAAGGTCGG	20	60	61.4	678
OTU42F (b)	CCTGAAAACGGCCCGCCT	18	66.7	60.52	648
OTU43F	GAAACGATGCTAATACCCCATATGCT	26	42.3	61.65	650
OTU44F	CCTTTAGGAAAGGGATACAATCGGAA	26	42.3	61.65	669
OTU52F	GCTCAGGAGGGAATAACGCTGA	22	54.5	62.12	670
OTU53F	CGCGGGAGGAAGGTTTTAGGA	21	57.1	61.78	412
OTU58F (a)	CCTTCAGGTTTGGGACAACCAC	22	54.5	62.12	669
OTU59F	GCTTCCAGTCGGGGATACAGTT	22	54.5	62.12	668
OTU63F	CTGCTTCCAGGTCGGGGATAA	21	57.1	61.78	672
OTU64	GGATGTAGGCCTCTGGGCT	19	63.2	60.98	410
OTU68	CCTTAGGAGGAGGATAACAGCT	22	50	60.25	668

OTU	Primer Sequence	Length	% GC	Tm	Amplicon
OTU71F	GGTTAATTCTGCCTAGGATGAGCT	24	45.8	61.01	608
OTU79F	CCGTAGGTCGGGGACAACA	19	63.2	60.98	668
OTU82	CGCTTTGAGCTAATAGTTCAAAGCCT	26	42.3	61.65	365
OTU83	CAGCTAGTTGGCGAGGTAAC	20	55	59.35	573
OTU84F (a)	GGATGTAAACTTCGCAAGTATGGGAA	26	42.3	61.65	399
OTU88	GGACGAAGGCTTACTGAGTTGTA	23	47.8	60.65	408
OTU89F	GGTTTATCGCCTGAAGATGAGCT	23	47.8	60.65	608
OTU90F	GTGAAAGGTTAATCGCCTGAAGGT	24	45.8	61.01	614
OTU91	GCTTCAGGTCGGGGACACACT	21	61.9	63.73	669
OTU92F	GGGATACAGAAGGAAACTACTGCT	24	45.8	61.01	658
OTU93	GTGAGGGAGGAAGGTTTTAGGACTGTAAACCA	32	46.9	68.21	699
OTU97F (d)	GAGATGGGCTTGCGGCTGAT	20	60	61.4	590
OTU98F	CCTCTAGGAAAGGGATACAATCGGAA	26	46.2	63.22	669
CYA781R(a)	GACTACTGGGGTATCTAATCCCATT	25	44	61.34	
CYA781R(b)	GACTACAGGGGTATCTAATCCCTTT	25	44	61.34	

B:

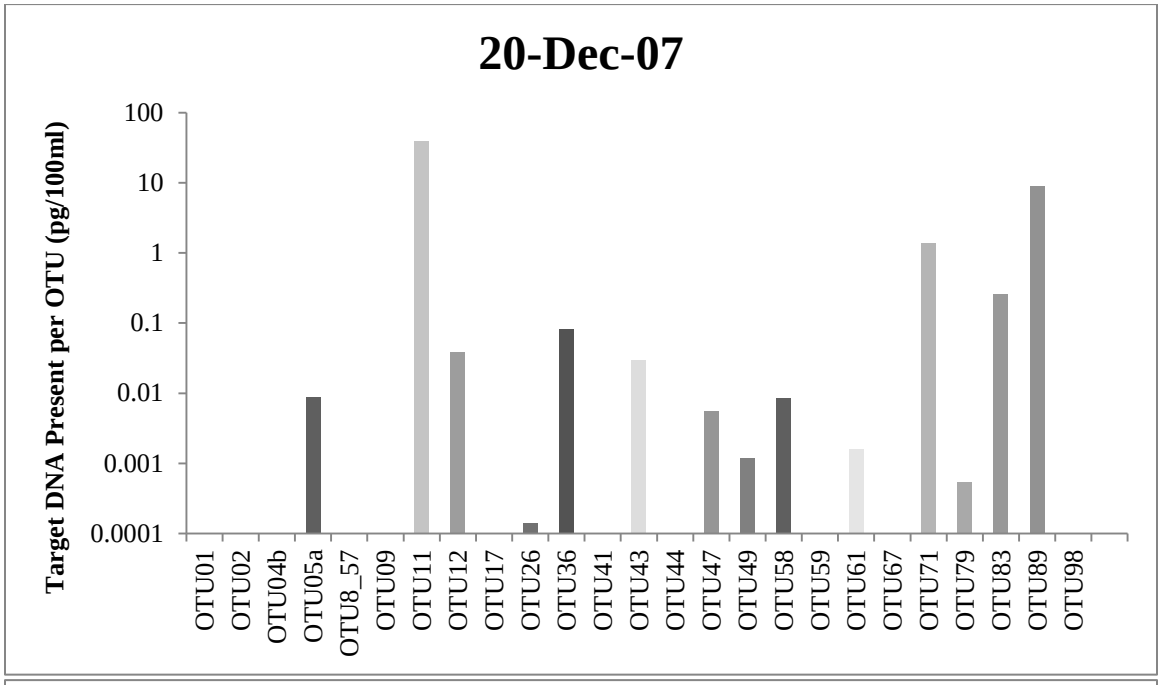
OTU	Primer Sequence	Length	% GC	Tm	Amplicon
F53-01/09/10/24/31/34/56/60/69/94	GGAAACGACTGCTAATACCCGATGT	25	48	62.98	
R431-01	GCCTACGAACGCTTTACGCCCAA	23	56.5	64.21	390
R293-09	GGYTTACAGCCCAGAGGCCTT	21	57.1	61.78	260
R388-10	GTGATTCCGGATAACGCTTGCATCCTCTGTA	31	48.4	68.17	367
R422-24	CACYTACAGACGCTTTACGCCCA	23	52.2	62.43	389
R294-31	GGTTTACGACCCAAGAGCCTT	21	52.4	59.82	261
R421-34	CCTGCGGACTCTTTACGCCCAA	22	59.1	63.98	389
R421-56	CCACCTACAGACCCTTTACGCCCAA	25	56	66.26	392
R419-60	GTTCCACCTGCAGACCCTTTACGCCCAA	28	57.1	69.51	393
R416-69	CCTACGGACGCTTTACGCCCAATGATT	27	51.9	66.49	389
R131-94	CCTTGGTAAGCCATTACCCTACCAA	25	48	62.98	102
F62-04a_74/14/16/29/30/45/65/78/81	GCTAATACTCTATATGCCGAGAGGT	25	44	61.34	
R292-04(a)-74	GGTTTACAACCCACAGGCTTTCAT	24	45.8	61.01	253
R292-14	GAGGTTTACAACCCACAGGCTGTCAT	26	50	64.8	254
R169-16	GCTGATCATCCTCTTAGACCAGCTA	25	48	62.98	130
R294-29	GAGGTTTACGACCCAAGAGCCTT	23	52.2	62.43	253
R402-45	CCAATAATTCCGGATAACGCTTGCAT	26	42.3	61.65	364
R412-30	CCTTTACGCCCAATCATTCCGGAT	24	50	62.72	372
R295-65	GAGGTTGACAACCCACAGGCCTT	23	56.5	64.21	254

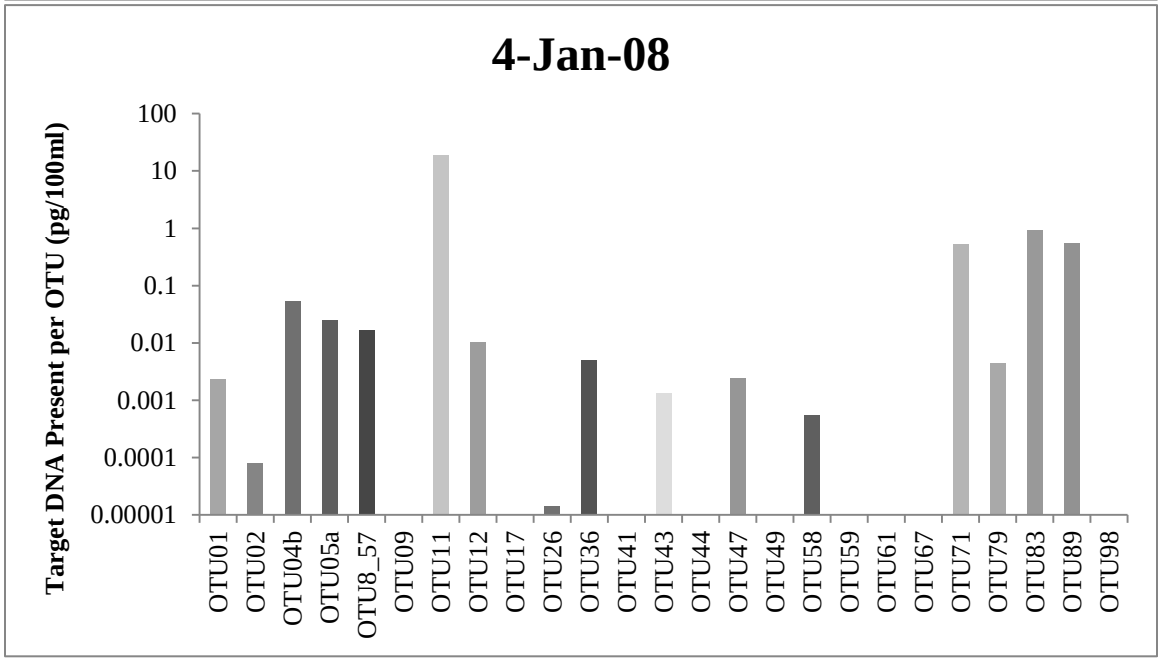
OTU	Primer Sequence	Length	% GC	Tm	Amplicon
R295-78	GAGGTTTACAACCCACAGGCCTT	23	52.2	62.43	254
R400-81	CCAATAATTCCGGATAACGCTCGCCT	26	50	64.8	362
F36-05a/05b/46/87/95	GGAAGAGAATACAATTGGAAACGGTTGCTA	30	40	64.03	
R289-05a	GGTTTACAACCCACAGGCCTTCATCCCT	28	53.6	68.05	280
R288-05b	GGTTTACAACCCACAGGCCGTCATCCCT	28	57.1	69.51	279
R288-46	GGTTTACAACCCATAAGGCAGTCATCCCT	29	48.3	66.68	280
R313-87	GTCAGTGCTTCTTCCCTGAGAAAAGTGGT	29	48.3	66.68	305
R313-95	GTCAGAGCTTCTTCCCTGAGAAAAGCGGT	29	51.7	68.09	305
F59-08_57/47/80	GTGGCTAATACCGAATGTGCCGA	23	52.2	62.43	
R409-57-08	CCCTTWACGCCCAATCATTCCGGATAA	27	48.1	64.97	373
R406-47	GCTTTACGCCCAATAATTCCGGATAA	26	42.3	61.65	371
R406-80	GCTTTACGCCCAGTGATTCCGGATAA	26	50	64.8	371
F30-07_96/67/76/100	GCCCTAGGAGGGGGACAA	18	66.7	60.52	
R297-07_96	GAGGTTTACAACCCTAAGGCCTT	23	47.8	60.65	289
R295-67	GAAGTTTACAATCCACAGACCGTCT	25	44	61.34	289
R297-76	GAGGTTTACAACCCACAGGCCTT	23	52.2	62.43	289
R288-100	CCAAGAGCCTTCCTCCCTCA	20	60	61.4	277
F30-17	CCTACAGACTCGGGGACAAAC	21	57.1	61.78	
R411-17	CCTTTACGCCCAATCATTCCGGA	23	52.2	62.43	424
F84-20/22_51/35/50	GGTGAAAGATTTATCGCCTGGAGAT	25	44	61.34	
R335-20	GGCTTATTCATCAAGTACCGTCAGA	25	44	61.34	274
R405-22_51	GCCCAGTGATTCCGGATAACG	21	57.1	61.78	340
R304-35	CCCTGAGAAAAGAGGTTTACAACC	24	45.8	61.01	242
R292-50	GCGGTTTACAGTCCTAAAACCTTC	24	45.8	61.01	230
F30-23	CCTACAGACTCGGGACACAG	20	60	61.4	
R408-23	GCTTTACGCCCAATAATTCCGGA	23	47.8	60.65	400
F30-61	CCTACAGACTCGGGACAACAGT	22	54.5	62.12	
R413-61	GCTTTATGCCCAGTGATTCCGGA	23	52.2	62.43	403
F81-19/49	GGAGGTGAAAAGAGTTTTGCCTA	23	43.5	58.87	
R293-19	GCGGTTTACAGTCCTAAAACCTT	23	43.5	58.87	234
R323-21/06/49	GCTACCGTCATTATCTTCACAGA	23	43.5	58.87	263
F30-53/86	GCCTTAGGAGGGGGACACA	19	63.2	60.98	
R349-53	GGAATTAGCCGATGCTTATTCCTCA	25	44	61.34	331
R303-86	CCAGAGAAAAGAGGTTTACAACCCTA	26	42.3	61.65	298
F92-21	GGTTAACTGCCTGAGGGTGA	20	55	59.35	
R323-21/06/49	GCTACCGTCATTATCTTCACAGA	23	43.5	58.87	252

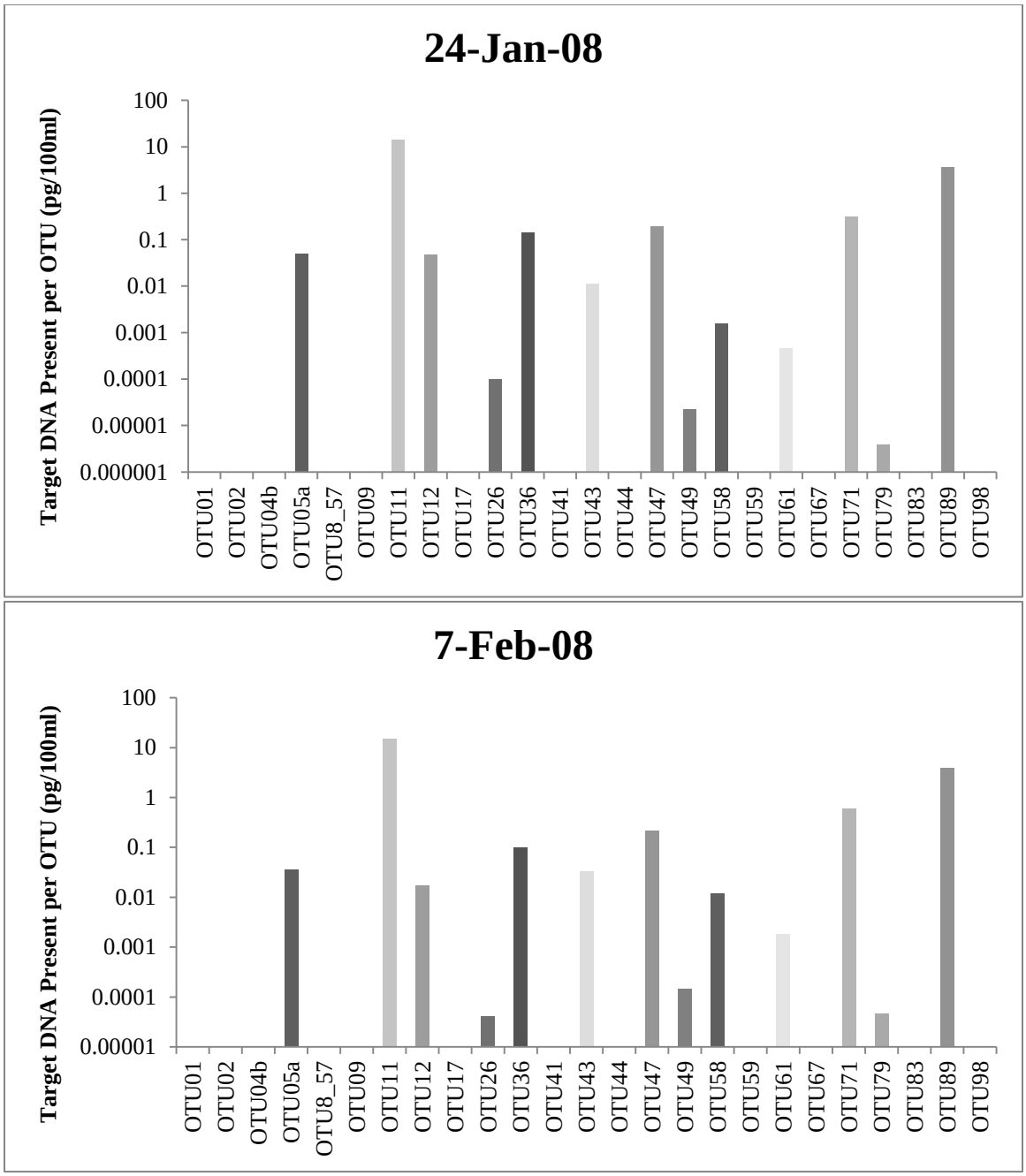
APPENDIX B:

OTUs PRESENT BY DATE

Cell counts calculated for each OTU by sampling date.



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10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
OTU17
OTU26
OTU36
OTU41
OTU43
OTU44
OTU47
OTU49
OTU58
OTU59
OTU61
OTU67
OTU71
OTU79
OTU83
OTU89
OTU98

7-Feb-08
Target DNA Present per OTU (pg/100ml)
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10
1
0.1
0.01
0.001
0.0001
0.00001
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
OTU17
OTU26
OTU36
OTU41
OTU43
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OTU71
OTU79
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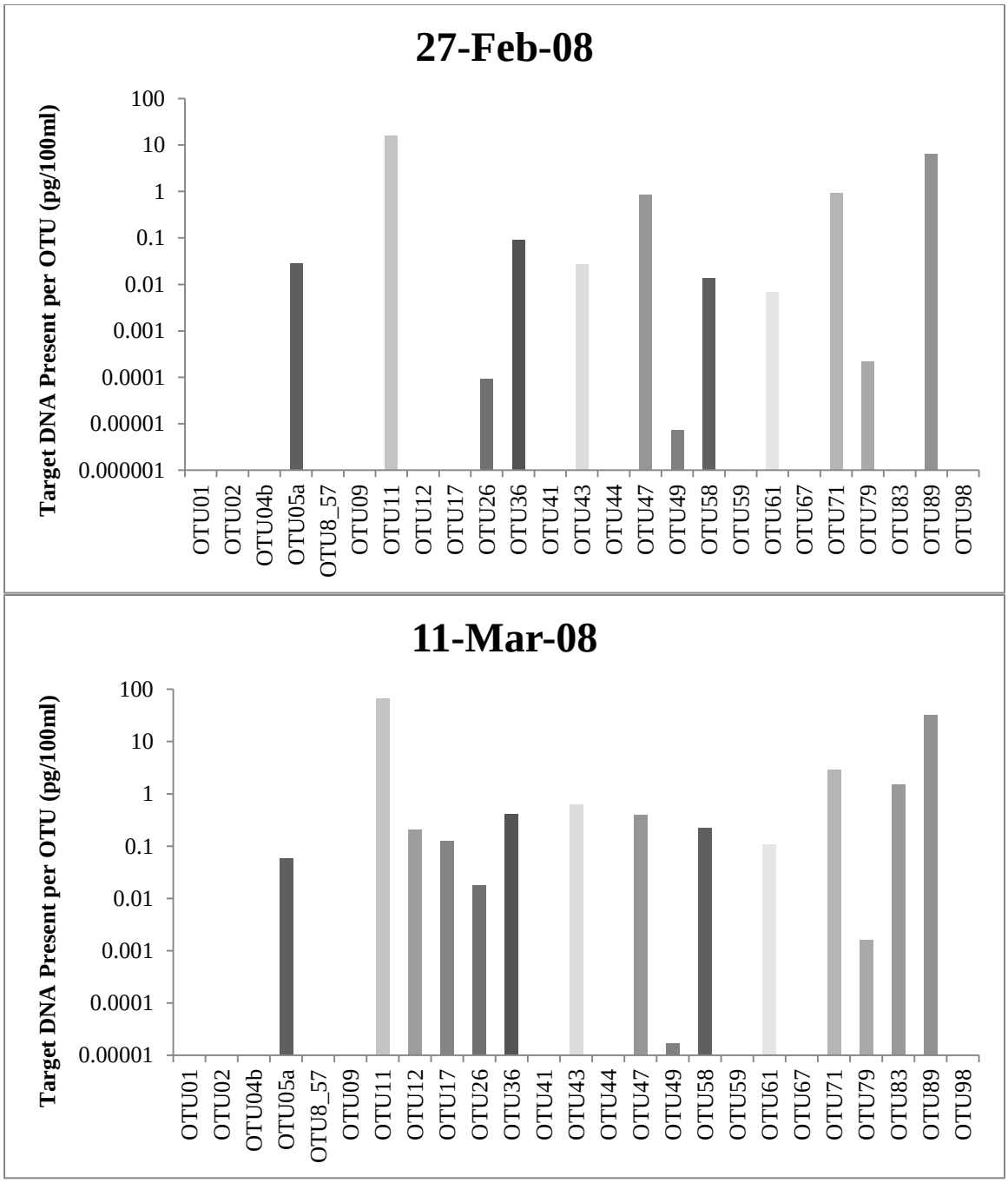
OTU89
OTU98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0.01
0.001
0.0001
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0.000001
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
OTU17
OTU26
OTU36
OTU41
OTU43
OTU44
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OTU83
OTU89
OTU98
11-Mar-08
Target DNA Present per OTU (pg/100ml)
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0.1
0.01
0.001
0.0001
0.00001
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
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OTU26
OTU36
OTU41
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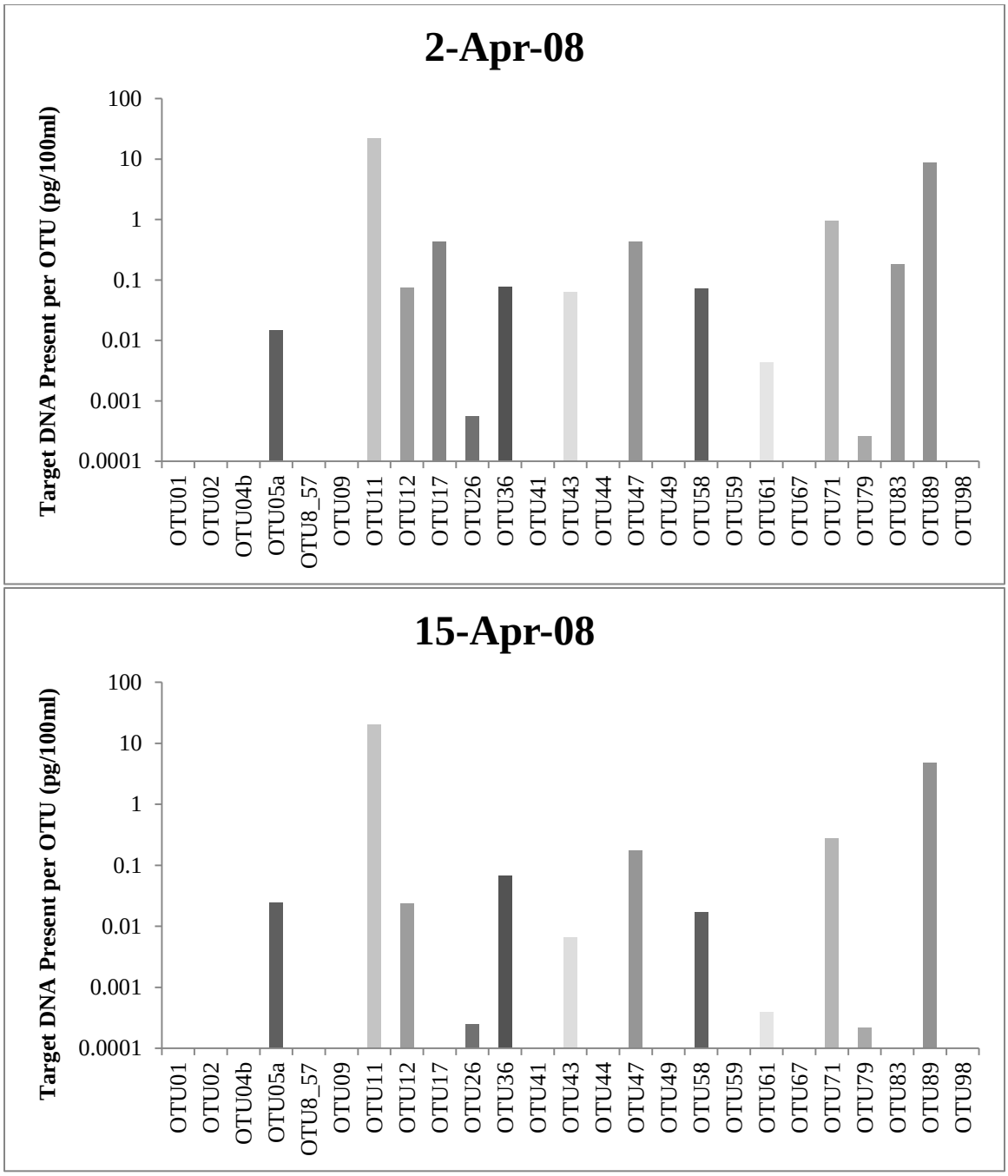
OTU98


2-Apr-08
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0.1
0.01
0.001
0.0001
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
OTU17
OTU26
OTU36
OTU41
OTU43
OTU44
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OTU79
OTU83
OTU89
OTU98
15-Apr-08
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0.01
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0.0001
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
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OTU36
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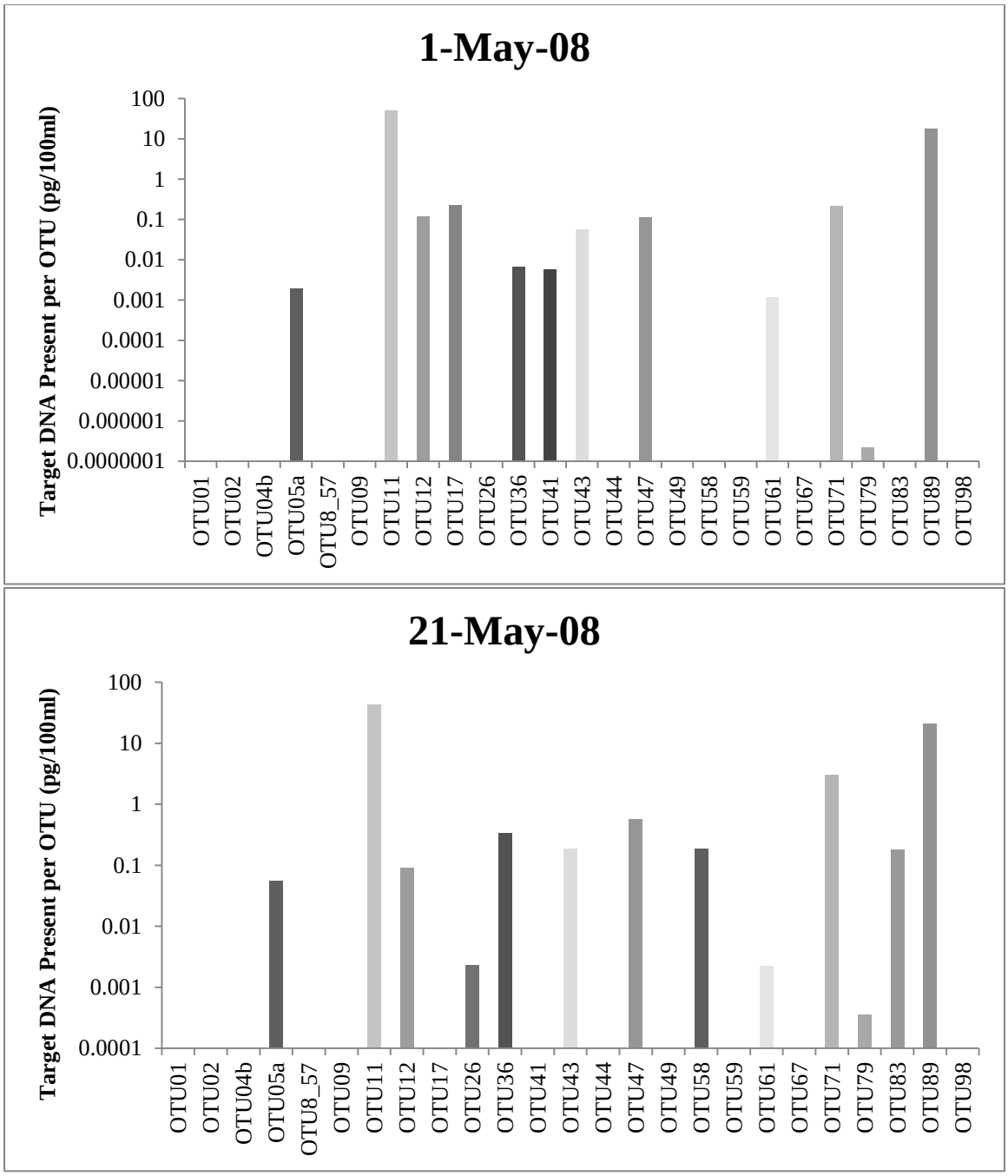
1-May-08
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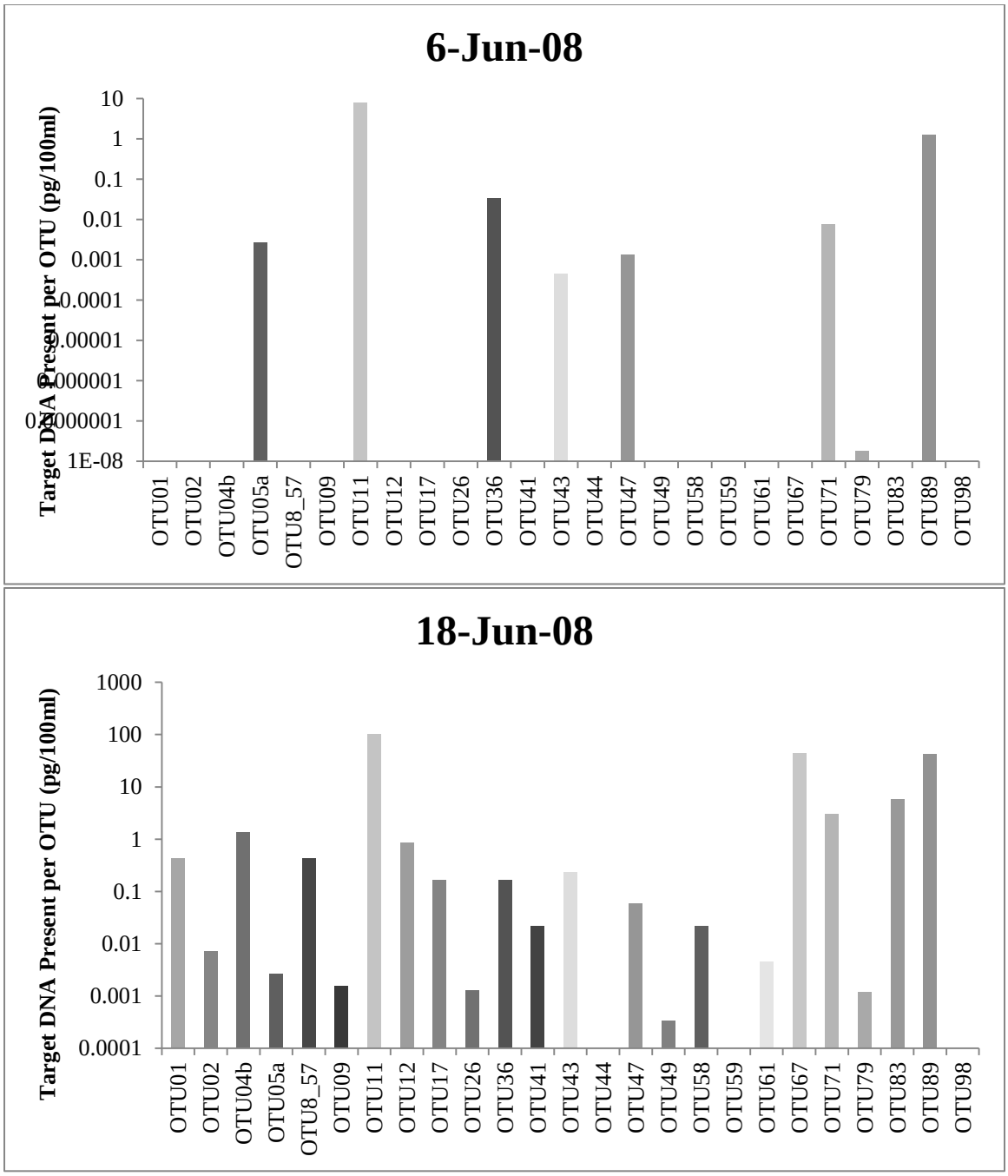
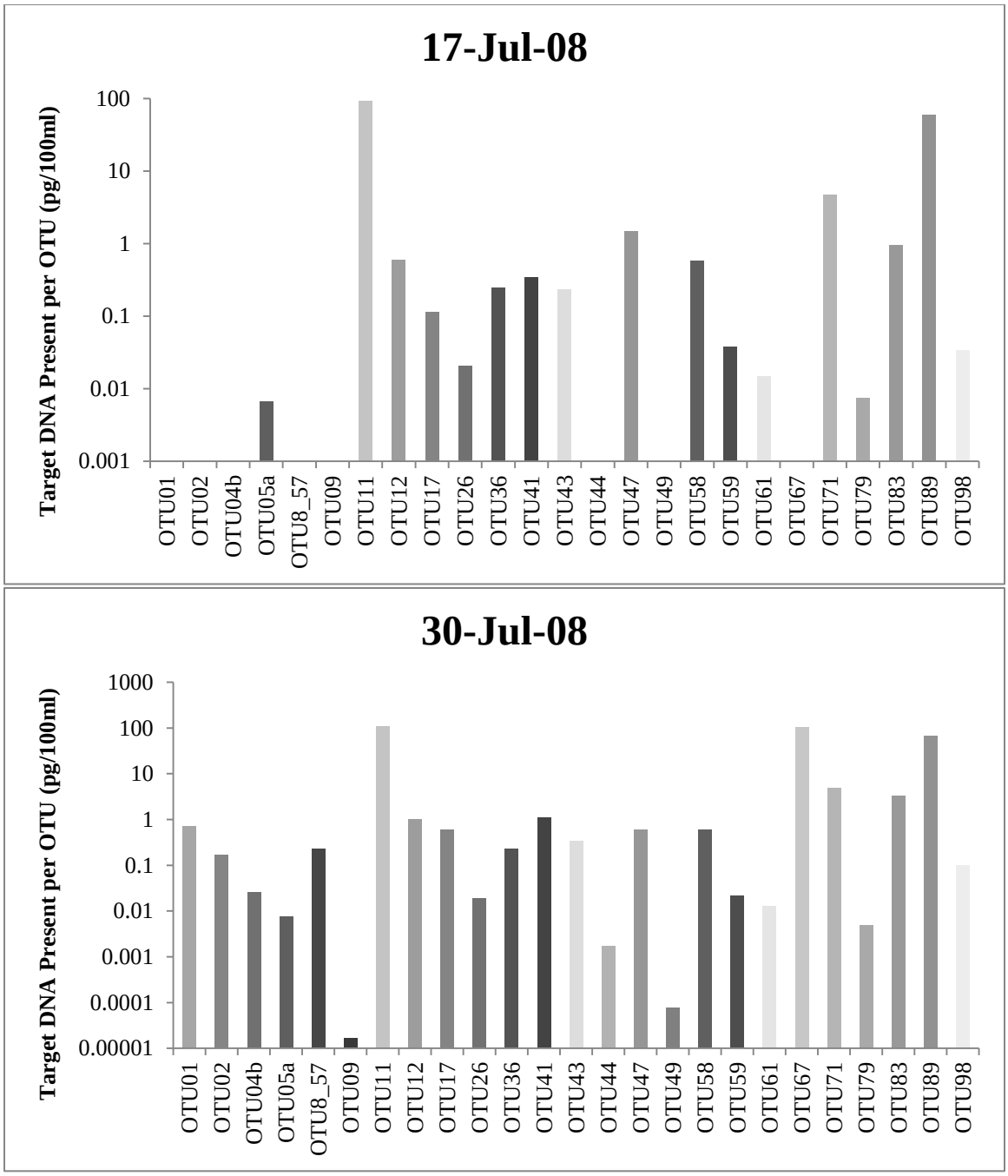
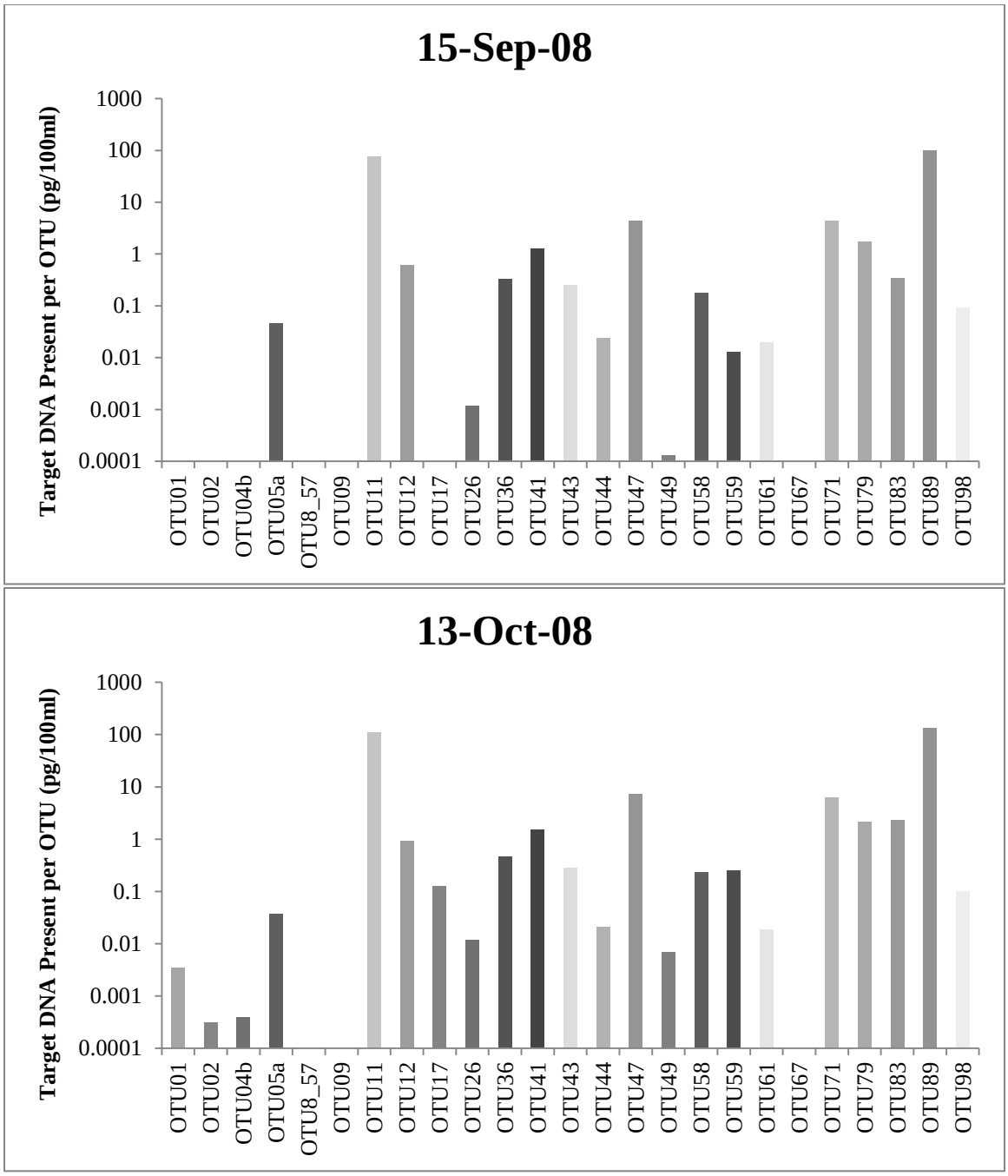
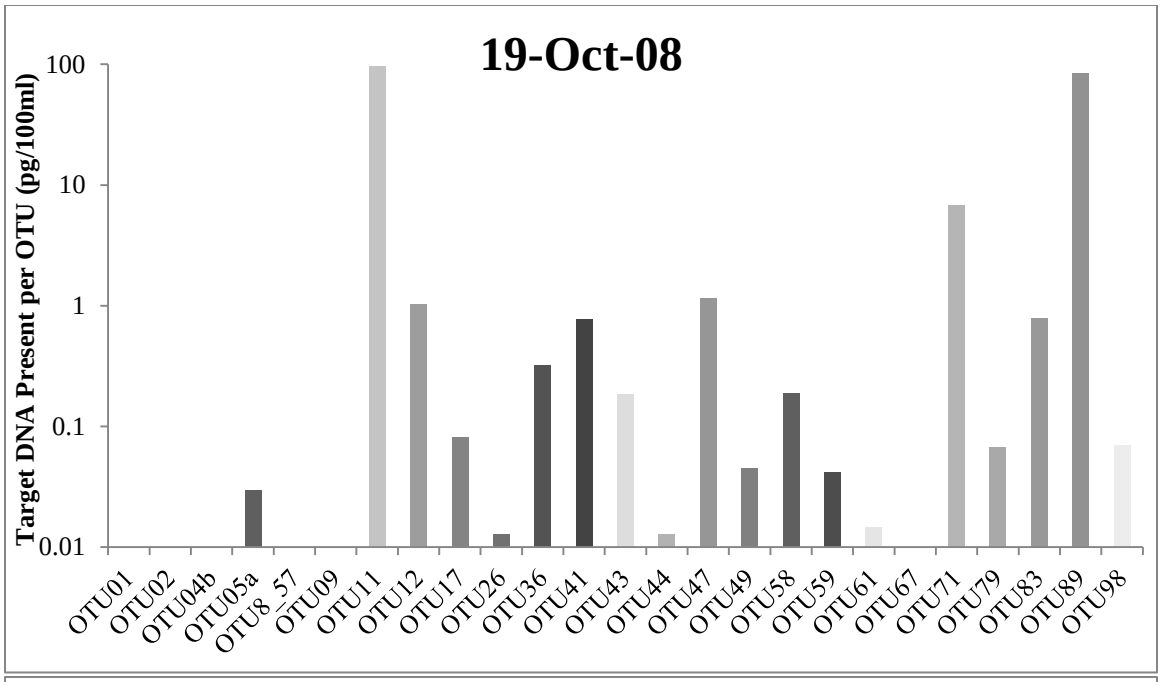
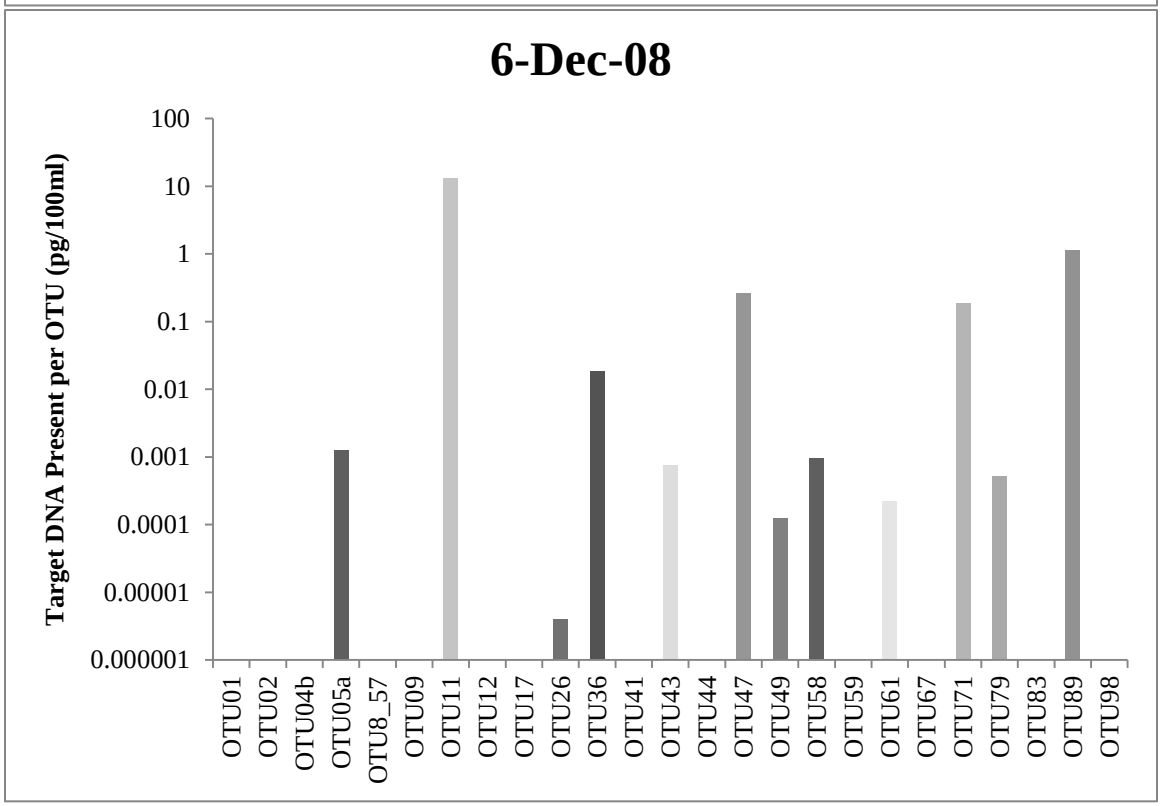
100
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
0.0000001
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
OTU17
OTU26
OTU36
OTU41
OTU43
OTU44
OTU47
OTU49
OTU58
OTU59
OTU61
OTU67
OTU71
OTU79
OTU83
OTU89
OTU98
21-May-08
Target DNA Present per OTU (pg/100ml)
100
10
1
0.1
0.01
0.001
0.0001
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
OTU17
OTU26
OTU36
OTU41
OTU43
OTU44
OTU47
OTU49
OTU58
OTU59
OTU61
OTU67
OTU71
OTU79
OTU83
OTU89
OTU98


6-Jun-08
Target DNA Present per OTU (pg/100ml)
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
0.0000001
1E-08
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
OTU17
OTU26
OTU36
OTU41
OTU43
OTU44
OTU47
OTU49
OTU58
OTU59
OTU61
OTU67
OTU71
OTU79
OTU83
OTU89
OTU98
18-Jun-08
Target DNA Present per OTU (pg/100ml)
1000
100
10
1
0.1
0.01
0.001
0.0001
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
OTU17
OTU26
OTU36
OTU41
OTU43
OTU44
OTU47
OTU49
OTU58
OTU59
OTU61
OTU67
OTU71
OTU79
OTU83
OTU89
OTU98


17-Jul-08
Target DNA Present per OTU (pg/100ml)
100
10
1
0.1
0.01
0.001
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
OTU17
OTU26
OTU36
OTU41
OTU43
OTU44
OTU47
OTU49
OTU58
OTU59
OTU61
OTU67
OTU71
OTU79
OTU83
OTU89
OTU98
30-Jul-08
Target DNA Present per OTU (pg/100ml)
1000
100
10
1
0.1
0.01
0.001
0.0001
0.00001
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
OTU17
OTU26
OTU36
OTU41
OTU43
OTU44
OTU47
OTU49
OTU58
OTU59
OTU61
OTU67
OTU71
OTU79
OTU83
OTU89
OTU98


15-Sep-08
Target DNA Present per OTU (pg/100ml)
1000
100
10
1
0.1
0.01
0.001
0.0001
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
OTU17
OTU26
OTU36
OTU41
OTU43
OTU44
OTU47
OTU49
OTU58
OTU59
OTU61
OTU67
OTU71
OTU79
OTU83
OTU89
OTU98
13-Oct-08
Target DNA Present per OTU (pg/100ml)
1000
100
10
1
0.1
0.01
0.001
0.0001
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
OTU17
OTU26
OTU36
OTU41
OTU43
OTU44
OTU47
OTU49
OTU58
OTU59
OTU61
OTU67
OTU71
OTU79
OTU83
OTU89
OTU98


19-Oct-08
Target DNA Present per OTU (pg/100ml)
100
10
1
0.1
0.01
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
OTU17
OTU26
OTU36
OTU41
OTU43
OTU44
OTU47
OTU49
OTU58
OTU59
OTU61
OTU67
OTU71
OTU79
OTU83
OTU89
OTU98


6-Dec-08
Target DNA Present per OTU (pg/100ml)
100
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
OTU01
OTU02
OTU04b
OTU05a
OTU8_57
OTU09
OTU11
OTU12
OTU17
OTU26
OTU36
OTU41
OTU43
OTU44
OTU47
OTU49
OTU58
OTU59
OTU61
OTU67
OTU71
OTU79
OTU83
OTU89
OTU98

APPENDIX C:

SPEARMAN RANK ORDER CORRELATIONS FOR INDIVIDUAL OTUs AND ENVIRONMENTAL VARIABLES

Cell Contents:
Correlation Coefficient
P-Value
Number of samples

The pairs of variables with positive correlation coefficients and P values below 0.050 tend to increase together. For the pairs with negative correlation coefficients and P values below 0.050, one variable tends to decrease while the other increases. For pairs with P values greater than 0.050, there is no significant relationship between the two variables. Correlations that are significant have been highlighted with red.

	TEMP	DO	PH	TURB	SS	TKN	TIN	TN	TP	TN:TP	AMMO	CHLA	TOC	Phyco
OTU01	0.329	-0.39	-0.352	0.106	-0.157	0.271	-0.205	0.0213	-0.153	0.245	0.282	-0.134	0.13	0.105
	0.178	0.108	0.148	0.668	0.524	0.27	0.407	0.928	0.541	0.321	0.252	0.592	0.603	0.674
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU02	0.329	-0.39	-0.352	0.106	-0.157	0.271	-0.205	0.0213	-0.153	0.245	0.282	-0.134	0.13	0.105
	0.178	0.108	0.148	0.668	0.524	0.27	0.407	0.928	0.541	0.321	0.252	0.592	0.603	0.674
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU04b	0.249	-0.336	-0.355	0.121	-0.103	0.225	-0.16	0.0695	-0.0376	0.137	0.259	-0.0981	0.0846	0.0527
	0.313	0.169	0.143	0.626	0.68	0.36	0.519	0.78	0.876	0.58	0.293	0.692	0.736	0.831
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU05a	-0.245	0.49	0.229	0.423	-0.158	-0.24	0.375	-0.106	0.0683	0.0382	-0.331	0.272	-0.441	-0.218
	0.321	0.0382	0.351	0.0774	0.524	0.33	0.122	0.668	0.78	0.876	0.175	0.27	0.0656	0.378
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU8_57	0.259	-0.361	-0.493	0.0302	-0.127	0.145	-0.0461	0.119	0.0207	0.0747	0.405	-0.273	0.266	-0.121
	0.293	0.138	0.0373	0.902	0.609	0.558	0.85	0.632	0.928	0.761	0.0925	0.266	0.281	0.626
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU09	0.506	-0.506	-0.455	-0.124	-0.23	0.35	-0.21	0.121	-0.22	0.327	0.471	-0.364	0.467	0.0385
	0.0314	0.0314	0.0564	0.621	0.351	0.151	0.397	0.626	0.374	0.181	0.0483	0.134	0.0494	0.876
	18	18	18	18	18	18	18	18	18	18	18	18	18	18

Cell Contents: Correlation Coefficient
P-Value
Number of samples

The pairs of variables with positive correlation coefficients and P values below 0.050 tend to increase together. For the pairs with negative correlation coefficients and P values below 0.050, one variable tends to decrease while the other increases. For pairs with P values greater than 0.050, there is no significant relationship between the two variables. Correlations that are significant have been highlighted with red.

	TEMP	DO	PH	TURB	SS	TKN	TIN	TN	TP	TN:TP	AMMO	CHLA	TOC	Phyco
OTU11	0.645	-0.467	-0.271	-0.338	-0.0742	0.476	-0.459	-0.0795	-0.278	0.315	0.331	0.0684	0.272	0.509
	0.00377	0.0494	0.27	0.167	0.767	0.045	0.054	0.748	0.259	0.198	0.175	0.78	0.27	0.0306
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU12	0.617	-0.408	-0.155	-0.294	-0.227	0.371	-0.425	-0.129	-0.278	0.291	0.268	0.0571	0.274	0.52
	0.0062	0.0908	0.53	0.231	0.356	0.127	0.0774	0.603	0.259	0.238	0.278	0.818	0.266	0.0269
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU17	0.5	-0.369	-0.355	-0.03	0.197	0.24	-0.286	-0.142	-0.0693	0.0181	0.266	-0.168	0.449	0.391
	0.0338	0.129	0.146	0.902	0.426	0.33	0.244	0.569	0.78	0.941	0.281	0.497	0.0602	0.106
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU26	0.521	-0.287	-0.34	-0.277	-0.207	0.312	-0.341	-0.132	-0.25	0.232	0.243	-0.0663	0.102	0.307
	0.0262	0.241	0.164	0.259	0.402	0.201	0.161	0.592	0.309	0.347	0.326	0.786	0.68	0.211
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU36	0.406	-0.131	0.0207	-0.109	-0.376	0.278	-0.259	-0.0795	-0.33	0.375	0.0196	0.234	-0.0715	0.255
	0.0925	0.597	0.928	0.656	0.12	0.259	0.293	0.748	0.178	0.122	0.934	0.343	0.773	0.301
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU41	0.666	-0.525	-0.0153	-0.395	-0.331	0.513	-0.56	0.0575	-0.435	0.478	0.224	0.193	0.179	0.707
	0.00245	0.0248	0.948	0.102	0.175	0.0291	0.0154	0.818	0.0699	0.044	0.365	0.436	0.471	0.000843
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU43	0.554	-0.333	-0.219	-0.135	-0.165	0.344	-0.251	-0.0547	-0.222	0.296	0.265	0.0684	0.234	0.353
	0.0169	0.172	0.374	0.586	0.508	0.159	0.309	0.824	0.369	0.227	0.281	0.78	0.343	0.146
	18	18	18	18	18	18	18	18	18	18	18	18	18	18

Cell Contents: Correlation Coefficient
P-Value
Number of samples

The pairs of variables with positive correlation coefficients and P values below 0.050 tend to increase together. For the pairs with negative correlation coefficients and P values below 0.050, one variable tends to decrease while the other increases. For pairs with P values greater than 0.050, there is no significant relationship between the two variables. Correlations that are significant have been highlighted with red.

	TEMP	DO	PH	TURB	SS	TKN	TIN	TN	TP	TN:TP	AMMO	CHLA	TOC	Phyco
OTU44	0.394	-0.222	0.271	-0.237	-0.361	0.197	-0.402	-0.218	-0.511	0.414	-0.017	0.245	-0.0816	0.609
	0.104	0.369	0.27	0.338	0.136	0.426	0.0962	0.378	0.0298	0.0856	0.941	0.321	0.742	0.00715
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU47	0.323	-0.0733	0.215	-0.174	-0.363	0.104	-0.348	-0.197	-0.537	0.358	-0.176	0.395	-0.274	0.56
	0.186	0.767	0.383	0.482	0.134	0.674	0.153	0.426	0.0211	0.141	0.476	0.102	0.266	0.0154
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU49	-0.0585	0.12	0.43	-0.164	-0.399	-0.0457	-0.224	-0.216	-0.512	0.312	-0.199	0.0576	-0.341	0.225
	0.811	0.626	0.0728	0.508	0.098	0.85	0.365	0.383	0.0291	0.205	0.421	0.818	0.161	0.36
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU58	0.536	-0.281	-0.247	-0.207	-0.22	0.316	-0.351	-0.135	-0.312	0.276	0.185	-0.0176	0.0517	0.375
	0.0217	0.252	0.317	0.402	0.374	0.195	0.148	0.586	0.201	0.263	0.456	0.941	0.831	0.122
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU59	0.521	-0.336	0.0444	-0.38	-0.369	0.357	-0.552	-0.0889	-0.487	0.396	0.0692	0.161	-0.0877	0.647
	0.0262	0.169	0.856	0.116	0.129	0.143	0.0173	0.717	0.04	0.102	0.78	0.514	0.723	0.00361
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU61	0.442	-0.142	-0.103	-0.149	-0.154	0.273	-0.21	-0.0217	-0.333	0.353	0.0764	0.152	0.0154	0.361
	0.0642	0.563	0.674	0.546	0.535	0.266	0.397	0.928	0.172	0.146	0.754	0.541	0.948	0.136
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU67	0.514	-0.514	-0.464	-0.114	-0.252	0.331	-0.198	0.0832	-0.258	0.353	0.482	-0.387	0.475	0.0308
	0.0283	0.0283	0.0516	0.644	0.305	0.175	0.421	0.736	0.297	0.146	0.042	0.11	0.045	0.902
	18	18	18	18	18	18	18	18	18	18	18	18	18	18

Cell Contents: Correlation Coefficient
P-Value
Number of samples

The pairs of variables with positive correlation coefficients and P values below 0.050 tend to increase together. For the pairs with negative correlation coefficients and P values below 0.050, one variable tends to decrease while the other increases. For pairs with P values greater than 0.050, there is no significant relationship between the two variables. Correlations that are significant have been highlighted with red.

	TEMP	DO	PH	TURB	SS	TKN	TIN	TN	TP	TN:TP	AMMO	CHLA	TOC	Phyco
OTU71	0.505	-0.261	-0.0971	-0.337	-0.281	0.298	-0.381	-0.162	-0.419	0.364	0.224	0.0808	-0.0275	0.404
	0.0321	0.289	0.692	0.167	0.252	0.224	0.116	0.514	0.0823	0.134	0.365	0.742	0.908	0.0943
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU79	0.329	-0.292	-0.064	-0.386	-0.277	0.174	-0.474	-0.221	-0.326	0.156	0.115	0.227	-0.162	0.511
	0.178	0.234	0.792	0.11	0.259	0.482	0.0461	0.369	0.183	0.53	0.644	0.36	0.514	0.0298
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU83	0.482	-0.424	-0.436	-0.224	-0.109	0.322	-0.341	-0.0713	-0.132	0.152	0.388	-0.0726	0.18	0.258
	0.042	0.0774	0.0684	0.365	0.662	0.186	0.161	0.773	0.592	0.541	0.108	0.767	0.466	0.297
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU89	0.67	-0.42	-0.0888	-0.398	-0.168	0.492	-0.463	-0.0723	-0.403	0.447	0.265	0.157	0.232	0.571
	0.00223	0.0806	0.717	0.0999	0.497	0.0373	0.0516	0.767	0.0943	0.0615	0.281	0.524	0.347	0.0133
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
OTU98	0.547	-0.393	0.0392	-0.333	-0.416	0.367	-0.495	-0.0915	-0.521	0.485	0.137	0.138	-0.0153	0.628
	0.0184	0.104	0.876	0.172	0.0839	0.131	0.0364	0.711	0.0262	0.041	0.58	0.58	0.948	0.00515
	18	18	18	18	18	18	18	18	18	18	18	18	18	18
Total	0.639	-0.454	-0.371	-0.205	-0.0169	0.539	-0.225	0.0728	-0.171	0.411	0.437	-0.0564	0.368	0.351
	0.00321	0.0497	0.114	0.393	0.94	0.0173	0.349	0.759	0.475	0.0791	0.0602	0.815	0.118	0.138
	19	19	19	19	19	19	19	19	19	19	19	19	19	19